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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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Vol. XXI. June, 1911. No. 1.

BIOLOGICAL BULLETIN

SOME REMARKS ON THE GASTRULATION OF DESMOGNATHUS FUSCA.

WILLIAM A. HILTON.

For the last three or four years I have been trying to secure material to complete observations made some time ago on the gastrulation of *Desmognathus*. There are many difficulties in the way of obtaining the right stages for this study, for the eggs are without pigment and enclosed in very tough membranes. Added to the difficulties of selection and orientation, is the fact that the eggs are large and not easily sectioned.

As mentioned in an earlier paper,[1] the eggs of this species are holoblastic although approaching the meroblastic type of segmentation. A moderate-sized blastula cavity is formed, above it, small cells with small yolk granules and below, large cells with larger granules. This cavity in later stages comes to be more or less filled in with larger or smaller cells which round off from the general mass (Figs. 1 and 2). To what extent the

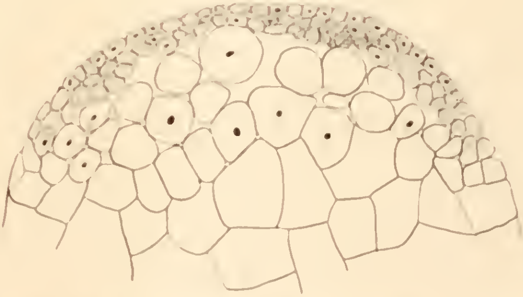

FIG. 1. Upper half of a late morula stage of *D. fusca*. The dotted cells are those with small yolk granules. × 25.

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[1] W. A. Hilton, "General Features of an Early Development of *Desmognathus fusca*," *Jour. Morph.*, Vol. XX., 1909.

segmentation cavity may in this way normally become filled in, I am at present unable to say, but I have found some specimens every year for the past few years in which the segmentation cavity was totally obliterated. As the cells divide, the small ones of the animal pole gradually extend about the larger yolk cells and, at an early stage, one may recognize at the point of junction of the two masses and in from the surface, some of the small cells which later go to form the mesoblast. These correspond to those recognized by Morgan, King, Ruffini and others.[1] At a stage when the small cells begin to migrate over the larger, there are about three layers of these with small yolk granules at the animal pole. This number later becomes reduced to two and then to one, similar to the single cell layer found by Lange in *Megalobatrachus;*[2] this outer layer or ectoblast becomes more extensive and may be found forming a simple definite covering over a large part of the egg. The other small cells, many if not all of which contain small yolk granules, have by this time migrated considerably, but not so extensively as the ectoblastic. Their exact limits are hard to determine. At about the time the ectoblastic covering at the animal pole has become reduced to one cell, the first indication of gastrulation was found. Fig. 3 is a section of a rather early stage.

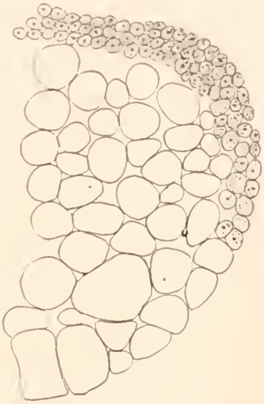
FIG. 2. Portion of a late morula stage of *D. fusca.* ×25.

As mentioned at an earlier time, the external appearance of gastrula stages do not differ from those of other amphibia to any great degree, but some details of development seem peculiar. I now have several series of earlier stages than have been available before and in the earliest of these, I find the gastrula mouth

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[1] T. H. Morgan, "The development of the Frog's Egg," 1897. H. D. King, "The Gastrulation of the Egg of *Bufo lentiginosus,*" *Am. Nat.*, Vol. XXXVI., 1902. A. Ruffini, "Contributo alla conoscenza della Ontogenesi degli Anfibi anuri ed urodeli," *Arch. It. Di. Anat. Di. Emb.*, 1907.

[2] D. de Lange, Jr., "Die Keimblätterbildung des *Megalobatrachus maximus* Schlegel," *Anat. Hefte*, Bd. 32, H. 3, 1907.

well down on the yolk mass and appearing as a cleft between the small cells which have grown down about the larger ones. This early cleft in all of my specmiens appears to be not entirely formed by invagination. In later stages, however, there is good evidence of ingrowth especially from the small cells of the upper lip. In a number of specimens of this stage, the rounded yolk cells which were in or near the segmentation cavity are more or less artificially crowded together, probably due to the loss of fluid between them in the passage through the alcohols, so that no clear idea was obtained of their positions. It is probable that about the time of gastrulation there is a considerable change of position of cells at the animal pole of the egg, the cells may have a looser arrangement, there may even be considerable areas between them occupied by fluid, evidence of which may be seen in certain living eggs. The loss of this fluid by fixation causes the collapse of the thin roof and the crowding together of the cells at the animal pole (Fig. 3). However, in many slightly later stages including both earlier specimens and those only recently obtained, the segmentation cavity seems to have been largely lost and still later than this, new spaces between the yolk cells make their appearance and communicate directly with

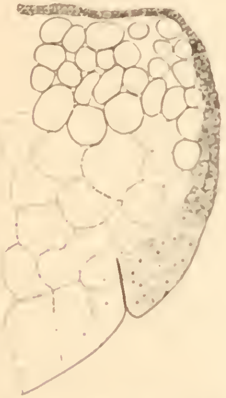

FIG. 3. The beginning of gastrulation of D. *fusca* indicated by a cleft. Specimen shrunken somewhat in the alcohols. ×25.

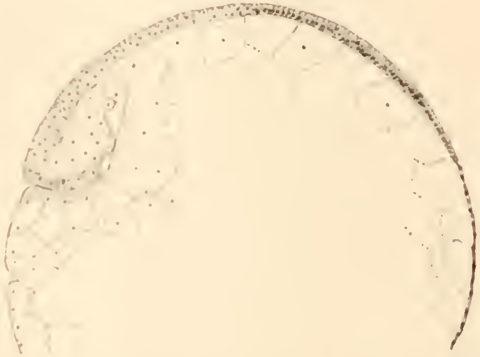

FIG. 4. Section through a later stage of gastrulation. Small cells have penetrated from the surface and a cleft between the cells is added. ×25.

the cavity formed by gastrulation. Fig. 4 is through a specimen of such a later stage where the total cavity, although slight, is clear and definite. The lining of the first part of this is from invaginated cells and some of the original ones which were with small yolk granules on the animal pole may be distinguished as part of the lining of the cavity, the total extent of which is shown in a few sections beyond this level.

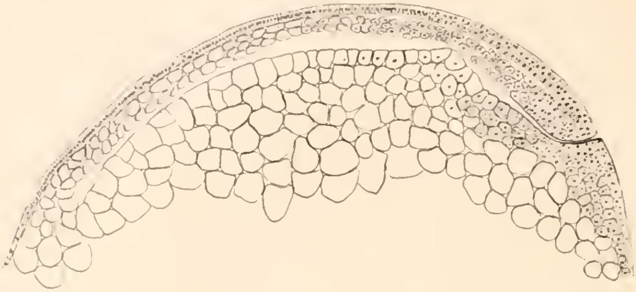

FIG. 5. Longitudinal section through blastopore and archenteron showing dorsal and ventral lip. Small cells with small granules dotted as in the other drawings. ×25.

In later stages the cavity or archenteron may not be very extensive. Cells just under the dorsal ectoblastic ones may be seen to be composed of small yolk granules and beyond the point where invagination is apparent these seem to be well organized and represent a part of the middle germ layer and were probably formed from some of the earlier small cells which were traced in their migrations about the yolk mass.

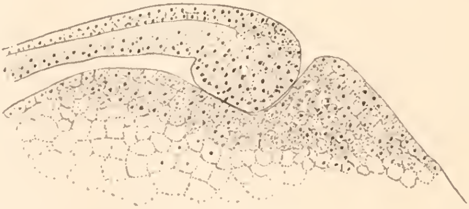

FIG. 6. Longitudinal section through the blastopore of a stage a little later than that of Fig. 5, showing cells which have grown in from the dorsal lip. ×25.

In other specimens the cavity of the archenteron is not very wide, most of the dorsal cells lining it contain small yolk granules, and these may represent some of the early small cells which have migrated about the yolk. Some of the cells near the opening of the blastopore have grown in dorsally and the ventral lip is also to some degree made up of small cells, some of which may be followed into the floor of the archenteron (Fig. 5).

In later gastrula stages as the enteron becomes more and more marked, there may be noticed a considerable ingrowth of cells, especially from the dorsal lip, in some specimens causing this free portion of the roof to become rolled as Brauer[1] found to be the case in the Gymnophiona (Fig. 6).

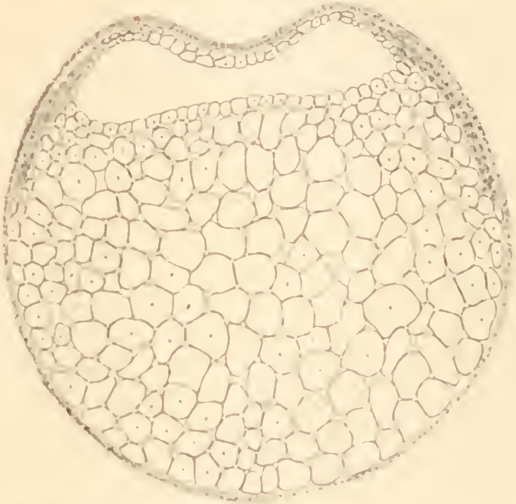

FIG. 7. Cross section through an embryo just before the beginning of the nervous system, showing the lateral extent of the mesoblast. ×25.

At a time when the blastopore is reduced to a very small hole and just before medullary folds are formed, the cavity has come to be large and extensive. Fig. 7 is from a cross section of such a stage. In it the mesoblast has not penetrated very far ventrally especially in the more cephalic region. Later than this, when the neural plate has begun to close, we find that as yet in the head end the mesoblast does not extend much farther but, in the caudal region, it has penetrated between the ectoblast and the yolk in all directions from a great mass of cells surrounding the blastopore, extending forward and to a slight extent back of it. This mass of cells, which is continuous cephalad with the nervous system and the mesoblast, is in this more caudal region an undifferentiated group. It is from this that the mesoblast grows out to meet that which has been formed on each side of the notochord (Fig. 8). The place where the mesoblast from the cephalic region and that from this caudal mass meet is often distinguishable because of a difference in the staining character of the cells. In this late stage when the body of the embryo is outlined, the cavity of the archenteron is much reduced in size (Fig. 8). This caudal mass is made up from cells on all sides of the blastopore, but especially cephalad of it and evidently represents in large part, the small cells or their progeny which with the narrowing of the blastopore have been brought to the dorsal side of the yolk mass.

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[1] A. Brauer, "Beiträge zur Kenntnis der Entwickelungsgeschichte und der Anatomie der Gymnophionen," *Zool. Jahrb. Anat. Abt.*, Bd. X., 1897.

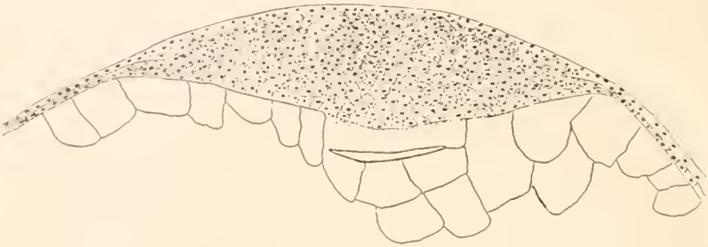

FIG. 8. Cross section through the caudal end of a late medullary plate stage, showing the undifferentiated cell mass, and the small size of the archenteron.

In the study of these stages in *Desmognathus*, one is impressed with the fact that fluids within the egg between the blastomeres have more significance than has been generally recognized and it seems possible that this fluid varying with the condition of development, may have considerable importance in the change of form and position of the cells. At times this liquid seems to exert no active influence on the size or condition of the cavities or shapes of the cells, and at others it seems to definitely force the cells into an even layer. For instance in early segmentation stages, those from the yolk mass and other surrounding cells round off toward the blastocœle and tend to fill it in, while at other times, as in late gastrula stages, the edges of the cells surrounding the archenteron are as smoothly pressed back from the lumen as though some definite substance occupied the large cavity.

The variations from time to time of the cavities within the egg are quite striking. The variations in extent of the segmentation cavity, its reduction and disappearance in some specimens at least, the development especially in the later stages of clefts between cells, the growth in extent of the archenteron and its reduction by the time the nervous system is developed; all of these changes may be dependent upon the large yolk mass and the peculiarities of environment. . If this last be a factor of importance in the cavity changes within the eggs, then we might expect to find, as we do, some variation in individuals of the same relative stage of development, for females with eggs or young are found in places that are from wet to almost dry at different seasons or during the same summer.

Some General Conclusions.

1. The small-granuled, small cells of animal pole grow about the larger with greater or less filling in of the segmentation cavity.

2. The cells of the animal pole become reduced to one layer.

3. The first indication of gastrulation appears in the vegetative hemisphere at a region between the small cells which have grown down and the yolk cells.

4. There is an ingrowth of small cells, from the dorsal lip especially.

5. The early segmentation cavity as such probably does not become joined to the invagination.

6. The later archenteron is formed partly by invagination and partly by separation between cells.

7. The cavity of the archenteron is apparently made up of invaginated cells, yolk cells and included under either one or both of these, some of the small cells which have migrated from the region of the animal pole.

8. The small cells at the animal pole form ectoblast.

9. The small cells at the animal pole at least some of which are under the surface cells form mesoblast and probably some entoblast.

10. In later stages of gastrulation there is a marked ingrowth from the dorsal lip of the blastopore.

11. Dorsal and ventral mesoblast become fused in later stages due to the formation of the caudal mass in front of and about the blastopore.

12. The caudal mass furnishes mesoblast in all directions. That formed caudally and laterally grows between the yolk cells and ectoblast. Later, the growing edges of the mesoblast fuse to enclose practically the whole yolk mass.

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Department of Histology and Embryology.

ECOLOGICAL SUCCESSION.

I. STREAM FISHES AND THE METHOD OF PHYSIOGRAPHIC ANALYSIS.

VICTOR E. SHELFORD.

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I. INTRODUCTION.

The writer is interested primarily in experimental work. In connection with experiments on the modification of taxonomic characters of the tiger beetles and the life-history work which necessarily accompanied it (Shelford, '08), certain striking relations of these beetles to environmental complexes were noted in 1905. These relations corresponded closely to the environmental relations and succession of plants, and illustrated the same principles as the plants themselves (Shelford, '07). Because of possible important bearings of the facts discovered on experimental work, and the mode of attacking the problems of evolution, the writer undertook to familiarize himself with the principles of physiography, and with the principles of plant ecology as set forth by Warming, Cowles, Schimper, Clements, Whitford, Livingston, Transeau, Flahault, Schröter, Moss, Schantz, Dacknowski and many others. The work of Adams and of other zoölogists has been followed in detail also.

It was my hope to secure guiding principles to be used in the interpretation of the tiger beetle data, in the fields where zoölogy has contributed only *chaos.* In 1907 an appointment requiring that I give instruction in natural history alone, using the field method, served to stimulate my efforts in this direction in order to find a basis for the organization of field and natural history instruction. In 1908, I was compelled, quite against my will, to drop experimental work for a time and have been left free to pursue the inquiry to its logical endings.

An early training in zoölogy which was of the strictest morphological type, caused me at the outset to share the doubts of many biologists as to the value of ecological work. However, because of the circumstances just referred to, I was able to examine the work of plant ecologists with a large degree of sympathy, which has grown as the inquiry and accompanying investigations have progressed. It will be seen that we have been *investigating the question of the value and relations of organized ecology to absorbing biological problems of today.* It is the results of this investigation that we are concerned in presenting here. The zoölogical investigations which have accompanied the work have been various and only the significant results will be presented. The investigations have been carried only far enough to indicate the lines in which they may profitably be directed further.

The result of our general inquiry, while in the main gratifying, is in some respects disappointing. We had hoped to find intimate relations between organizable ecology and the absorbing biological problems of the day, but everything points to the fact that animal ecology must be organized independently first, and related to other problems after organization has been attained. For this reason and for the sake of clearness, we have separated ecology as sharply from other subjects as possible.

It should be noted at the outset that the basis, in principle, of modern ecology has been developed by botanists quite independently of other divisions of the subject of botany. Doubts as to the value of plant ecology once existed among botanists, but these have disappeared and ecology has a recognition on a level with evolution, morphology, and physiology. This is of interest here, because history often repeats itself.

In general, animal ecology is concerned with the relation of animals to their environments. The *first essential is to locate the animal in its environment.*

Our inquiry and the papers which will deal with the results have centered around the following purposes:

1. To determine the animal activity which takes place within the narrowest limits and which is most important to the species, with a view to locating motile animals in their environments.

2. To determine the phenomena in animals which correspond to growth-form in plants. Growth-form in plants is usually a convenient index of the physiological conditions within.

3. To determine the validity of ecological succession as applied to animals.

4. To determine the value of the principles of physiography and plant ecology, (*a*) in locating animals in their environments, (*b*) in determining something of the physiological character of the organism as a whole, and (*c*) in the analysis of the organism.

5. To inquire into the possibility of organizing a system of ecological classification for animals which shall be sufficiently independent of the other systems and methods, to form a basis for generalization which may serve as a check on the results of the fields of evolution, heredity, and physiology.

6. To inquire into the relation of succession to the quantitative problems of biology.

7. To inquire into the relation of succession to the origin of adaptations.

8. To inquire into the relations of ecology to the economic problems of zoölogy, and the possibility of using ecology as a means of bringing about a better unification of the various branches of zoölogy, pure and applied.

It has been found impracticable to organize the work so that one paper will deal with the subject of any one of the above propositions alone. The discussion of physiological animal geography in the Whitman Memorial Volume of the *Journal of Morphology* is intended to serve as an introduction to the general questions and deals mainly with the first two and the fifth purposes. The present paper, which is intended to be the first of a series of five or six, is concerned with the question of the value of the principles of physiography as outlined in the fourth.

With the development of the ideas of genetic physiography came the recognition of the succession of physiographic conditions over a given locality (point *B* of Fig. 1). The relations of plants and animals to physiographic features being recognized, the first recognition of plant and animal succession came in connection with physiographic succession. Cowles ('01) carried out a complete classification of the vegetation near Chicago on the basis of the plant succession which accompanies physiographic change. Adams ('01) also discussed in general geographic terms the relations of animals to base-leveling and stream development. He referred to succession of forms in streams. Since his paper, little has been done in the study of the actual relations of animals to the various stages of stream development, or the relative importance of the activities of the animals and the dynamics of streams in determining distribution.

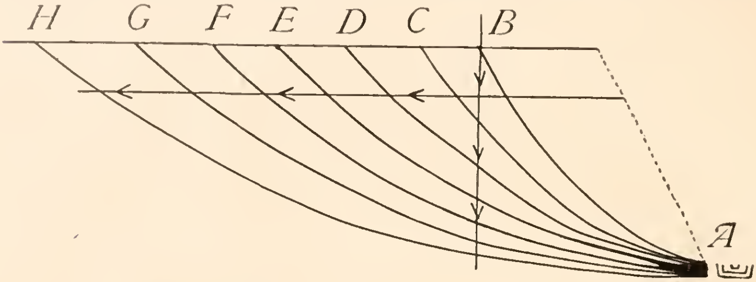


FIG. 1. A diagram showing the successive stages in the profile (general shape of the bottom) of a very young stream, curved lines, *A–B*, *A–C*, *A–D*, *A–E*, *A–F*, *A–G*, *A–H* representing the successive profiles. The uppermost horizontal line represents the surface of the land into which the stream is eroding. The horizontal line with the arrow heads indicates the direction of the migration of the source of the stream and accordingly of similar stream conditions. The vertical line with arrow heads when followed downward passess through a succession of stream conditions and represents physiographic succession at the locality *B*. The point *A* is the mouth of the stream. Opposite this are shown three successive sizes of the stream. and therefore succession at that point. The vertical scale is greatly exaggerated.

In the study of all phases of distribution, animal activities have been usually either ignored or taken for granted. Taxonomy has been the center around which all such work has rotated and the taxonomic characters used have been very generally structural. In the study of succession this has been true, only to a lesser degree. We believe that workers in this line have had in mind the recognition of activities, but the subject is too new to make such an ideal realizable in practice.

It is the purpose of this paper to present some details of the distribution of fish which shall throw light on the limitations and applications of the principles which Adams set forth.

The presentation of data and the discussion are based on the following questions:

1. Are the fish of the headwaters of older streams the same as the fish of younger streams?

2. If so, did they get into the stream when it was young and simply keep pace with the advance of erosion into the land?

3. What is the relation of the activities of animals to their distribution in streams?

4. What aspects of succession are of purely local and what are of general significance?

II. Localities and Material Studied.

In the investigation of problems in any field, one of the things requiring great care is the selection of material. In the study of ecology, if it is to be concerned with particular groups, this is true, and in addition a *still more important selection must be made*, namely, *that of the localities for study.*

1. *The Material.*

For this study fish were selected because they are probably the only animals that are not introduced by accidental means. They must have entered the streams studied at their mouths.

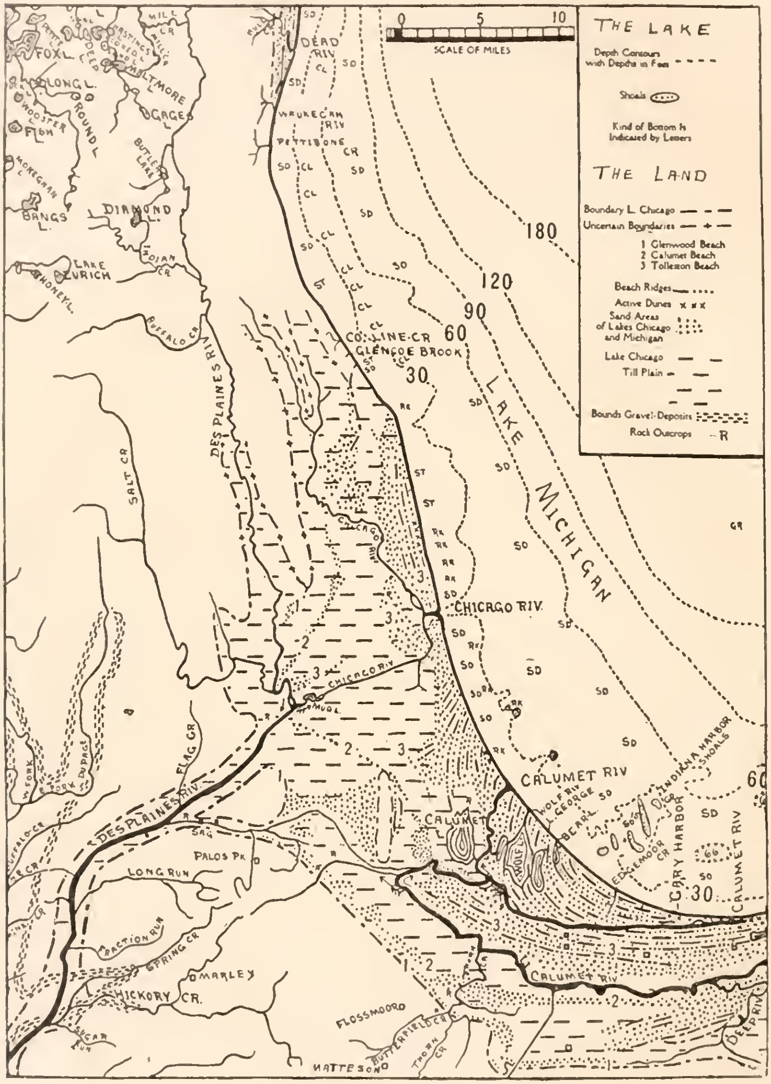
2. *The Points of Study.*

For the purposes of this study, the fish of several small streams, within forty miles of Chicago, have been collected. These streams are all indicated on the map.

(*a*) *Present Conditions.*

Beginning with the most northerly on the map, the streams are:

Bull Creek-Dead River.—It extends inward about three fourths of a mile from the boundary of old Lake Chicago (see map), and divides into two main branches, each of which has a number of tributaries. The total distance from the old Lake Chicago bluff to the headwaters is about two and one fourth miles. Its lower


THE LAKE
Depth Contours with Depths in Feet
Shoals
Kind of Bottom is Indicated by Letters
THE LAND
Boundary L. Chicago
Uncertain Boundaries
1 Glenwood Beach
2 Calumet Beach
3 Tolleston Beach
Beach Ridges
Active Dunes
Sand Areas of Lakes Chicago and Michigan
Lake Chicago
Till Plain
Bounds Gravel-Deposits
Rock Outcrops
R
0
5
10
SCALE OF MILES
FOX L.
LONG L.
ROUND L.
WOOSTER L.
FISH L.
DEEP L.
HASTINGS L.
MILL CR.
MALTMORE L.
GAGE L.
BUTLER LAKE
MONEGHAN L.
BANGS L.
DIAMOND L.
INDIAN CR.
LAKE ZURICH
HONEY L.
BUFFALO CR.
DES PLAINES RIV.
SALT CR.
DEAD RIV.
WAUKEGAN RIV.
PETTIBONE CR.
CO. LINE CR.
GLENCOE BROOK
CHICAGO RIV.
CHICAGO RIV.
CALUMET RIV.
WOLF RIV.
L. GEORGE
BEAR L.
WOLF L.
CALUMET L.
EDGEMOOR CR.
CARY HARBOR
INDIANA HARBOR SHOALS
CALUMET RIV.
CALUMET RIV.
DEEP RIV.
LAKE MICHIGAN
180
120
90
60
30
60
30
66
SD
CL
ST
RK
GR
DESPLAINES RIV.
FLAG CR.
DUPAGE
SAG
PALOS PK.
LONG RUN
FRACTION RUN
SPRING CR.
MARLEY
HICKORY CR.
SUGAR RUN
FLOSSMOOR
BUTTERFIELD CR.
THORN CR.
MATTESON
1
2
3

Map of the Vicinity of Chicago.

course which was added by the falling of the water of the lake, is known as Dead River.

Pettibone Creek is similar to Bull Creek, but a little smaller. Its length does not exceed one and three fourths miles.

County Line Creek is about twelve miles farther south. It differs from the others in being smaller. It also divides into two branches at a distance of only three sixteenths of a mile from its mouth and the length (with tributaries) is about one half mile.

Glencoe Brook is about one mile south of County Line Creek and has a total available length, so far as fish are concerned, of only a few rods.

These four streams are closely comparable. They all rise in a moraine which stands between 60 and 100 feet above the lake. Its height at the lake, or point nearest the lake, is about 60 feet in every case. With the exception of the lower courses of Bull Creek and Pettibone Creek, all are composed of strictly intermittent riffles and permanent (except in unusual seasons) pools. We will refer to these as the North Shore streams. In addition to these, two other streams were studied for comparison—Thorn-Butterfield Creek and Hickory Creek, including its west branch.

Hickory Creek is very different from the others. It drains a number of marshes and is permanent. Its upper course is larger than that of the north shore streams; it is sluggish and meanders through prairie marshes with only a very slight fall. The level of this part of the stream represents that of a depression between glacial ridges (Goldthwait, '09). So far as the present cycle of erosion is concerned (Salisbury, '07), erosion has proceeded as far as Marley. Below this point the stream is a typical swift brook.

Thorn-Butterfield Creek is intermediate between Hickory Creek and the north shore streams. The southern branch of Butterfield Creek, near Matteson, Ill., is sluggish, but has intermittent riffles and permanent pools.

(*b*) *History of the Region.*

When the Wisconsin ice sheet retreated from its maximum extent, which lay beyond the center of the state of Illinois, the edge of one of its lobes (Atwood and Goldthwait, '08) took up a position a few miles outside of, and parallel with, the present shore of Lake Michigan. Here it deposited what is known as the Valparaiso Moraine. When the ice retreated from this position, it occupied the basin of Lake Michigan a little to the north of the present south end of the Lake. Water occupied the space between this lobe and the Valparaiso Moraine. This body of water is known among geographers and geologists as Lake Chicago. At its period of maximum extent (see map), it stood 55 to 60 feet above the present lake. The history of Lake Chicago and the other predecessors of Lake Michigan is complicated

TABLE I.

SHOWING THE DISTRIBUTION OF FISH IN THE NORTH SHORE STREAMS AT THE TIMES INDICATED. The numbers refer to Fig. 2 and Fig. 4.

Name of Stream and Common Name of Fish.	Date and Scientific Name.	1	2	3	4	5	6	7
Glencoe Brook	August, 1907							
Horned dace	*Semotilus atromaculatus*	*						
County Line Creek	1907–8.							
Horned dace	*Semotilus atromaculatus*	*	*	*	*			
Blacknosed dace	*Rinichthys atronasus*			*	*			
Johnny darter	*Boleosoma nigrum*			*	*			
Blackhead minnow	*Pimephales promelas*				*			
Bluntnosed minnow	*Pimephales notatus*				*			
Common sucker	*Catostomus commersonii*				*			
Pettibone Creek[1]	September, 1909, and April, 1910.							
Horned dace	*Semotilus atromaculatus*	?	*	*	*			
Red bellied dace	*Chrosomus erithrogaster*		*	*	*			
Blacknosed dace	*Rinichthys atronasus*			*	*			
Johnny darter	*Boleosoma nigrum*			*	*			
Common sucker	*Catostomus commersonii*				*			
Bull Creek-Dead River	September, 1909.							
Horned dace	*Semotilus atromaculatus*	*	*	*	*	*		
Red bellied dace	*Chrosomus erithrogaster*		*	*	*			
Blacknosed dace	*Rinichthys atronasus*			*	*			
Common sucker	*Catostomus commersonii*				*	*		
Bluntnosed minnow	*Pimephales notatus*					*	*	
Little pickerel	*Esox vermiculatus*					*	*	*
Blue gill	*Lepomis pallidus*						*	*
Large mouthed black bass	*Micropterus salmoides*						*	*
Pike	*Esox lucius*							*
Crappie	*Pomoxis annularis*							*
Red horse	*Moxostoma aureolum*							*
Chub sucker	*Erimyzon sucetta*							*
Golden shiner	*Abramis crysoleucas*							*
Common shiner	*Notropis cornutus*							*
Cayuga minnow	*Notropis cayuga*							*
Tadpole cat	*Schilbeodes gyrinus*							*

[1] The lower part of Pettibone Creek has been destroyed by the U. S. Naval School otherwise the table would include the records for a point 5 and perhaps a point 6, but probably not 7. ? indicates incomplete identification.

and it will suffice, for our purposes, to state that the level of the water fell to a point about 40 feet above the present Lake Michigan. After standing in this position for a time, it fell again to a point 20 feet lower. Its next period of standstill was at a 12-foot level from which it receded to the present lake level.

The oldest of the north shore streams probably began as mere gullies when the lake was at its 60-foot level. These gullies have worked their way back into the morain and deepened their channels in the manner described by Adams ('01) and diagrammatically illustrated in Fig. 1. Long ago Bull Creek was similar to the present County Line Creek. At a recent period County Line Creek was also like Glencoe Brook.

There have been some modifications of these processes caused by changes in lake level. Each time the level of the lake fell, the streams were left with a steep portion near the mouths. Under

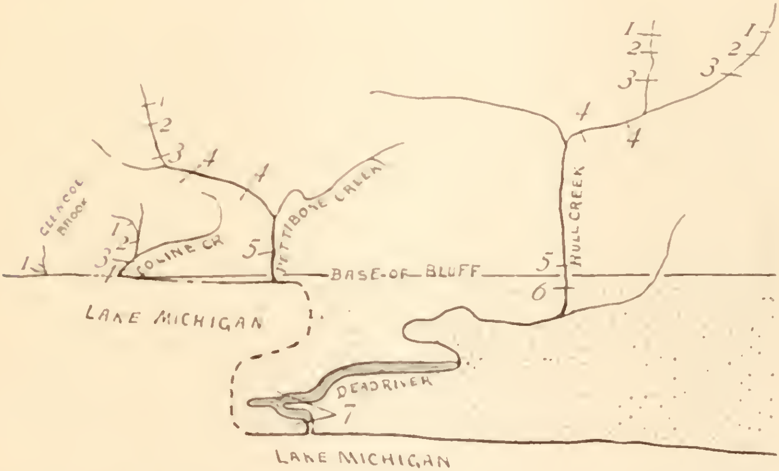


FIG. 2. Diagrammatic arrangement of the north shore streams. The streams are mapped to a scale of one mile to the inch and the maps are placed as closely together as possible in the diagram. The intermediate shore-lines are shown in broken lines which bear no relation to the shore lines which exist in nature. Toward the top of the diagram is west.

Each number on the diagram refers to the pool nearest the source of the stream which contains fish, as follows; 1, the horned dace (*Semotilus atromaculatus*); 2, the red-bellied dace (*Chrosomus erythrogaster*); 3, the black-nosed dace (*Rinichthys atronasus*); 4, the suckers and minnows; 5, the pickerel and blunt-nosed minnow; 6, the sunfish and bass; 7, the pike, chub, sucker, etc. The bluff referred to is about 60 feet high. The stippled area is a plain just above the level of the lake.

such conditions the steep portions migrate back into the moraine, *i. e.*, up stream, until the source is reached. The process of lowering the water level at the mouth of a stream and the development of the steep portion is known as rejuvenation. Rejuvenation usually affects the distribution of fishes in streams.

However, streams which are made up of intermittent pools and riffles are not so much affected as larger streams. Furthermore, softness of the glacial clay in which the north shore streams are located would cause the changes in the stream bed to take

TABLE II.

THE DISTRIBUTION OF FISH IN HICKORY CREEK (AND ITS WEST BRANCH) IN THE SUMMER OF 1909.

Those starred were in the pool nearest the source. I., The first mile of the stream, measured from the fish pool nearest the source, toward the mouth; II., the third and fourth miles; III., at the head of erosion, five miles from the pool nearest the source; IV., six miles from the pool nearest the source; V, nine miles from same; stream much larger with good riffles and one weedy cove.

		I.	II.	III.	IV.	V.
Horned dace*	*Semotilis atromaculatus*	*	*	*	*	*
Golden shiner*	*Abramis crysoleucas*	*	*		*	
Johnny darter*	*Boleosoma nigrum*	*	*	*	*	
Stone roller*	*Campostoma anomalum*	*	*	*		*
Straw colored minnow*	*Notropis blennius*	*	*		*	*
Blue spotted sunfish*	*Lepomis cyanellus*	*	*	*		*
Blunt nosed minnow	*Pimephales notatus*	*	*	*	*	*
Common sucker*	*Catostomus commersonii*	*	*			*
Mud minnow	*Umbra limi*		*			
Top minnow	*Fundulus notatus*		*	*		
Red bellied dace	*Chrosomus erythrogaster*		*	*		
Chub sucker	*Erimyzon sucetta*		*		*	
Black bullhead	*Ameiurus melas*		*			*
Black fin	*Notropis umbratilis*			*	*	
River chub	*Hybopsis kentuckiensis*			*	*	*
Fan tailed darter	*Etheostoma flabellare*			*	*	*
Rainbow darter	*Etheostoma cœruleum*	*		*	*	*
Least darter	*Microperca punctulata*				*	
Sucker mouthed minnow	*Phenacobius mirabilis*				*	
Cayuga minnow	*Notropis cayuga*				*	*
Rock bass	*Ambloplites rupestris*				*	*
Common shiner	*Notropis cornutus*					*
Rosy faced minnow	*Notropis rubifrons*					*
Banded darter	*Etheostoma zonale*					*
Blue gill	*Lepomis pallidus*					*
Long eared sunfish	*Lepomis megalotis*					*
Stone cat	*Noturus flavus*					*
Yellow perch	*Perca flavescens*					*
Small mouthed black bass	*Micropterus dolomieu*					*
Hogsucker	*Catostomus nigricans*					*
Common red horse	*Moxostoma aureolum*					*

place rapidly. In the existing streams the slope of bed differs within a given stream and with respect to the different streams. The differences do not appear to be of importance in determining the relations of the fish present to a stream as a whole. Accordingly, we shall not consider the effect of rejuvenation in the discussions following.

III. Presentation of the Data.[1]

Data of the kind upon which this paper is based are doubtless familiar to all collectors of fish. From our point of view the presence or absence of a given species is not of particular importance, but we are concerned with the *arrangement* of fish in the streams and its causes. Such causes cannot be discussed here because they should be studied experimentally. As yet we have not been able to do this.

Fig. 2 shows the streams and Table I. the distribution of the fish in these streams.

1. *Discussion of Table I.*

We note that the fish (horned dace) of the smallest stream (Glencoe Brook) belong to the same species as those at the headwaters of the larger streams. The red-bellied dace, when present, is not found so far upstream as the horned dace. The red-bellied dace is absent from County Line Creek. Since all the fish in all the streams must have entered from Lake Michigan, one is

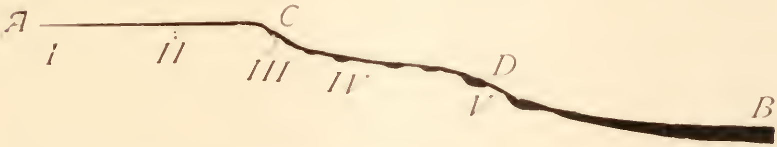


Fig. 3. Diagrammatic profile of Hickory Creek. *A*, source; *B*, mouth; *C*, head of erosion; *D*, rock outcrop. The figures below refer to the columns in Table II. and represent parts from which fish were collected.

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[1] The fish of County Line Creek were collected and their arrangement studied from time to time between 1907 and 1910. The arrangement of the fish as shown by the map of the stream was about the same throughout the periods of study, and Table I., except for the drought of 1908. The collections from the other streams were made in the summer and autumn of 1909, partly with the assistance of Mr. S. F. Hildebrand, of the Field Museum of Natural History.

surprised that they have found so many of the streams as is indicated here. The absence of the red-bellied dace is no doubt due to accident.

The black-nosed dace is present in all the streams except Glencoe Brook, but lower down than the two just mentioned. It is usually accompanied by the Johnny darter, which is absent from Bull Creek. This absence is probably due to the same causes as the absence of the red-bellied dace from County Line Creek. The Johnny darter probably had never become established in County Line Creek; only two specimens have been taken and opportunities to secure a complete collection were good when the pools dried.

Records of the occurrence of the blunt-nosed and blackhead minnows in County Line Creek are based on a single specimen of each taken by a student from the pool nearest the mouth of the stream. We have taken no others and these were probably not residents.[1] We note that at the points marked 4 (Fig. 4) as shown in column 4, all the fishes occurring nearer the source of the stream are present and in addition the young of the common sucker.

In Bull Creek at point 5, as shown in column 5, the daces are represented by the horned dace alone; the other two being absent. The minnows were present here in numbers and evidently regular residents. The little pickerel was not abundant.

In columns 6 and 7 (see map also), we find the record of a number of fishes belonging to large creeks and to ponds. The distribution of the fishes in Table I. corresponds to the habitat preferences indicated by Forbes and Richardson ('08), pages cix to cxiii.

2. *Discussion of Table II.*

By comparing columns 1 to 6 in Table I. with column I. of Table II., we note that nearly all the fish present in the north shore streams are present at the point nearest the source of Hickory Creek. Considering the first, third and fourth miles of this stream we note that, the little pickerel and the black-nosed dace are absent in the preërosion part of Hickory Creek and present in the north shore streams. In addition, we find several species in Hickory Creek (among these is a single specimen of darter, the species probably not resident) not found in the north shore streams at all. Hickory Creek is better situated for fish to enter, as they may come upstream directly, while in the north shore streams they must enter the lake and enter a given stream by chance. Accordingly, the larger number of species is to be expected in Hickory Creek.

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[1] Hankinson has pointed out the difficulties of securing a complete collection of fish. He sees fish in the water which he has difficulty in securing with a dip net.

In column III. are shown the fishes at the head of erosion or the riffles nearest the source of the stream. A number of swift-water fish—darters—are shown here. These were abundant.

In column IV. the occurrence of a number of fishes similar in habits to those shown in column III. is indicated. The stream is similar in bottom and volume of water at the localities represented in columns III. and IV., and further collecting would probably have shown these two localities to be inhabited by practically the same fish. The rock bass was represented by a single juvenile specimen.

Column V. shows the fish at a point in the stream where the volume of water is about four times that at point IV. The stream here is characterized by riffles and large pools. This was more thoroughly studied. Collections were made several times during the season and daily for nearly a week in the month of September, 1908. Here was taken a single juvenile specimen of the horned dace. The red-bellied dace and Johnny darter were not taken. The addition of a number of larger fishes here is of interest. These additional species are characteristic of large streams.

3. *Discussion of Table III.*

Table III. is introduced to show conditions intermediate between the north shore streams and Hickory Creek. The localities of study were selected to correspond to localities in Hickory Creek, just so far as conditions in Thorn-Butterfield Creek would permit. In general, conditions at A in this stream correspond to I. in Hickory Creek, but differ in that the water at A is confined to pools in dry weather and is continuous at I. B corresponds to III. and C to V.

An inspection of column A shows that some of the same fishes are present as in the uppermost pool of Hickory Creek. Again there are certain differences. These differences are the absence of certain fish—the Johnny darter, the golden shiner, the straw-colored minnow and the common sucker. The presence of these at locality I. of Hickory Creek is probably due to the artificial exposure of bare bottom.

Table III.

The Fish of Thorn Creek Collection Made at the Headquarters in 1908 and 1909 and at Other Points in 1909 and 1910.

A, The first fish pool; B, four miles down stream; C, ten miles down stream.

		A	B	C
Horned dace	*Semotilus atromaculatus*	*	*	*
Blunt nosed minnow	*Pimephales notatus*	*	*	*
Blue spotted sun-fish	*Lepomis cyanellus*	*	*	*
Stone roller	*Campostoma anomalum*	*	*	*
	Notropis umbratilis		*	?
Banded darter	*Etheostoma zonale*		*	?
Common shiner	*Notropis cornutus*		*	?
Striped top minnow	*Fundulus dispar*		*	?
Black sided darter	*Hadropterus aspro*		*	*
Johnny darter	*Boleosoma nigrum*		*	*
Mud minnow	*Umbra limi*		*	*
Cayuga minnow	*Notropis cayuga*		*	*
Golden shiner	*Abramis crysoleucas*		*	*
Large mouthed black bass	*Micropterus salmoides*			*
Small mouthed black bass	*Micropterus dolomieu*			*
Blue gill	*Lepomis pallidus*			*
Crappie	*Pomoxis sparoides*			*
Pirate perch	*Aphredoderus sayanus*			*
Yellow perch	*Perca flavescens*			*
Carp	*Cyprinus carpio*			*
Black bull head	*Ameiurus melas*			*
Common sucker	*Catostomus commersonii*			*
Short-headed red horse	*Moxostoma breviceps*			*
Pike	*Esox lucius*			*

Comparison of this list with that of the north shore streams (except 6 and 7) shows that, in so far as the north shore species are in Thorn-Butterfield at all, they are at the headwaters with the exception of the Johnny darter.

In column B we note that the fish are about the same species as were found in localities III. and IV. in Hickory Creek, in so far as they have been found in both streams at all. *Notropis cornutus* is found further upstream in Thorn-Butterfield Creek than in Hickory Creek.

In column C we note a number of fishes, twenty species and four doubtful identifications, as against twenty-two at point V. in Hickory Creek. Ten of these are the same in the two streams. Again we find larger fishes in the large parts of the stream.

IV. Discussion of the Data.

It is easy to lose oneself in the maze of taxonomic diversity which one finds in a number of streams separated no further than the ones considered here. We are not concerned with taxonomy. The suggestions and inferences herein are chiefly to illustrate a method of analysis and to suggest a method of combined experimental and field study. It should be borne in mind throughout the discussion that ecology as an organized science is in much the same state as morphology was when it was necessary to discuss methods which are now taken for granted.

1. *Distribution of Fishes in the Streams.*

We will consider the north shore streams first. We have noted that they are similar to each other in origin, in materials eroded, in their relations to the lake, and in being for the most part made up of permanent pools and intermittent riffles. An inspection of Table I. shows that there is a definite arrangement of fishes in these streams. Furthermore, that there is a close correspondence between the upper courses of the different streams in the matter of the fishes present, as well as in their arrangement. The *only* species, the horned dace, in the youngest stream (Glencoe Brook) is the same as the species which is *nearest the source of all the other streams.*

There are some differences in the fish communities of corresponding parts of the different streams, but these are probably due to the failure of a given species to enter a given stream. One is struck with the similarity of the corresponding fish communities rather than the differences.

In making a general comparison, reference to the diagram of the maps of the streams (Fig. 2) shows that the different *fish communities* are *closer* together in the smaller streams. Fig. 1 shows that the slope of the bed of the young streams is greater than that of the older streams, and the different *conditions* accordingly closer together.

Turning to the other two streams studied (Hickory Creek and Thorn-Buttterfield Creek), we see that the same species, the horned dace, is at the headwaters of these, as at the headwater of the north shore streams. It is accompanied, however, by several other species, a part of which are found in the north shore streams at points further down stream and in a larger volume of water than some individuals of the horned dace. In other words the species of the north shore streams are crowded together in streams where the volume of water is greater at the headwaters than in the north shore streams.

(*a*) *Causes of the Definite Arrangement of the Fishes in the Streams.*

The arrangement of the fish in these streams suggests definite reactions to some factor or factors in the stream. Rheotaxis is suggested as a cause of the upstream movement, and water pressure and size of stream a factor limiting the upward movement. This should be studied experimentally.

(*b*) *Origin of the Fish Communities.—Migration.*

A discussion of the mode of origin of the fish communities is concerned with the mode of entrance of the fish into the habitat. While manner of entrance of fish into a stream is not of particular importance to us, it follows from a reference to Fig. 1 that fish may enter when the stream is young and keep pace with erosion. Fish entering when a stream was at the youngest stage indicated in Fig. 1, need only maintain their position against the current and they would be carried inland as the source of the stream migrated inland. In the case of Bull Creek, the horned dace is absent from the lower portion (Dead River) so far as our collections show. Did this species enter when Bull Creek was similar to Glencoe Brook? This is improbable for the condition in Bull Creek, when it was similar in size to Glencoe Brook, must have been similar to conditions now found near glaciers, *e. g.*, Greenland.

There is further evidence as to the improbability of such early entrance in the data of Hickory Creek fishes. We have noted that

Hickory Creek rises in a depression between two morainic ridges, and that its upper course is sluggish and did not originate by stream erosion. Its profile is shown in Fig. 3, page 19.

The species of fish now at the source of this stream could not have entered when this stream was young and have kept pace with the migration of conditions, because if this was a stream at all during the close of the glacial epoch, it was carrying the waters from melting ice or a pond in the same manner as it is now draining a marsh.

The fishes or species of fish that are now found near the source of this stream were obliged to travel a distance of five miles through a mucky marshy stream to reach the headwaters. The success of most of the species present in the sluggish upper course of this stream is probably due also to local artificial exposure of gravel, since the settlement of the country. There has been dredging to facilitate drainage. Six of the thirteen species are known to use bare bottom for breeding. Forbes and Richardson show that of the ten of these which they considered in their tables (pp. cix-cxiii) nine show a preference for the bottom of rock and sand. Some of these preferences are very decided.

This shows that fish may enter the headwaters of streams without particular reference to physiographic conditions. Here positive rheotaxis may be a factor again (Lyon, '04).

The observations of the effect of drought and flood throw further light on the cause and rate of migration.

(*c*) *The Effect of Drought and Flood on the Arrangement of Fishes in Streams.*

1. *Droughts.*—There was an unusual drought in the autumn of 1908. The data on the distribution of fishes in Glencoe Brook and County Line Creek were collected before this date. Table IV. shows the arrangement *after* the drought.

County Line Creek was entirely dry except the pool nearest its mouth in September, 1908. This is the locality marked 4 in Fig. 2. The following spring was one of normal rainfall. *The fish proceeded upstream a distance of only three rods.* This partially restored the usual arrangement. If this represents the rate, fish proceed upstream slowly. Glencoe Brook has not recovered its fish.

TABLE IV.

LOCALITIES ON THE BASIS OF THE DISTRIBUTION OF FISH BEFORE THE DROUGHT. (SEE TABLE I.)

Name of Stream and Common Name of Fish.	Date and Scientific Name.	1	2	3	4
Glencoe Brook—no fish	October, 1909.				
County Line Creek.	September, 1909.				
Horned dace	*Semotilus atromaculatus*			*	*
Black nosed dace	*Rinichthys atronasus*				*
Common sucker	*Catostomus commersonii*				*

2. *Floods.*—Abbott ('70) collected the fishes from source to mouth of a small stream. After a flood he found none of the same fish but species belonging to larger streams. Unfortunately, he does not state that he again studied the stream from source to mouth, and accordingly leaves the question of a general upstream migration unsettled.[1]

2. *Succession.*

Ecologists are frequently asked what is meant by succession and what is the significance of succession. In answering the first part of the question there has often been a confusion resulting from a lack of distinction between the different uses of the term succession. It is used in three distinct senses. We speak of (1) *ecological succession*, (2) *seasonal succession*, and (3) *geological succession* (Adams, '09).

1. Geological succession is primarily succession of species throughout a period or periods of geological time. It is due

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[1] As evidence of upstream migration of mollusca, the following seems to be important. Frequent examination of a section of the North Branch of the Chicago River at Edgebrook, between 1903 and 1907, showed that *Pleurocera elevatum* and *Campeloma* occur in this stream. *Pleurocera* was not found during this period (ending November, 1907) above a certain point. *Campeloma* was found only sparingly above this point. The spring of 1908 was one of heavy rainfall and the streams were in flood from April to June. On July 6 the snail *Pleurocera* was found in numbers one fourth of a mile further upstream, than formerly. *Campeloma* had gone nearly as far. The season from November to April was not different from other seasons and there is no reason to assume that the migration began before the spring floods. If this is true the snails could make their way toward the headwaters at the rate of at least a mile per year, if they were introduced into a large stream. This must be a response to both water pressure and current. The small value of such single observations is recognized, but they are presented here because the opportunity to secure such data is small.

mainly to *the dying out of one set of species and the evolution of others which take their places.*

2. Seasonal succession is the succession of species or stages in the life-histories of species, over a given locality, due to *hereditary and environic differences in the life-histories* (time of appearance) of the species living there.

3. Ecological succession is the succession of ecological types (physiological types, modes of life) over a given point or locality, due to changes of environmental conditions at that point. From this point of view *we have nothing to do with species except that names* are necessary. There are of course relations between these three phenomena but these relations are of the nature of checks in method of study rather than essentials, and space will not permit us to discuss them here. There are also qualifications of a similar nature that might be made to the definitions. With reference to the statement that species are irrelevant to the question, a word should be added. If the habits of a species are a part of the definition of that species, as they must sooner or later come to be, then species are significant. The definition applies to the manner in which the species question is treated in practice.

We have noted the migration aspect of the relation of fish in small streams. Succession is but a different point of view. Succession of fish means succession of established forms, *i. e.*, of forms that make the stream their regular abode. The most important aspect of establishment is breeding. A number of writers have discussed the breeding habits of the species found in these small streams (Eigenmann, '95; Forbes and Richardson, '08; Hankinson, '07, '10; Reighard, '08; Reeves, '07; Smith, '08).

(*a*) *Statement of Ecological Succession.*

Succession in the case before us is a reconstruction. It is based on the superposition of all the fish communities over the oldest part of the oldest and largest stream. To make this clearer, we will state with the aid of the diagram (Fig. 4) the succession of fish in Bull Creek. This succession will be considered as taking place over the oldest part of the portion of Bull Creek which lies back of the bluff of the former and higher levels of Lake Michigan. This is the point designated as 5.

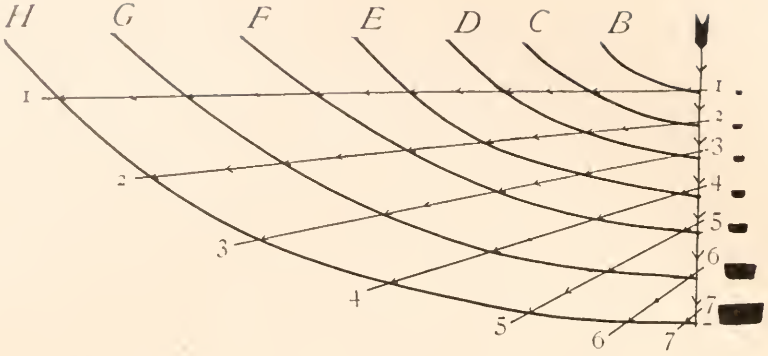

FIG. 4. This figure is based on Fig. 1. The profiles of the streams shown there are here separated vertically at the mouth. The curved lines represent seven stream stages as follows: *B*, Glencoe Brook; *C*, hypothetical stage; *D*, hypothetical stage; *E*, County Line Creek; *F*, Pettibone Creek; *G*, hypothetical stage; *H*, Bull Creek-Dead River. The hypothetical stages could, no doubt, be found along the shore of Lake Michigan, the difficulty arises from the introduction of sewage into so many streams.

The comparative size of the mouth of each stream stage is represented by a stream cross-section at the right.

The direction of reading in succession is indicated by the vertical line with the arrow heads pointing downward. The oblique lines marked 1–1, 2–2, 3–3, etc., pass through points in the stream profiles which are in the same physiographic condition, and which are occupied by similar fish communities.

When Bull Creek was at a stage represented by the first stage in our diagram (which is now represented by the present Glencoe Brook) its fish, if any were present, were ecologically similar to those now in Glencoe Brook in their relations to all factors except climate. This ecological type is represented by the horned dace alone. As Bull Creek eroded its bed and became hypothetical stage C of the diagram, the fish community of stage B was succeeded by a fish community *ecologically similar* to the fish communities at the localities marked 2 in Figs. 2 and 4. The fish now ecologically representing this community are the horned dace and the red-bellied dace. The community of the single species, the horned dace, had at such a period moved inland to the point where line 1–1 crosses the curved line representing the profile of hypothetical stage C.

As erosion continued the fish community ecologically represented by the horned dace and red-bellied dace moved gradually inland and was succeeded by a fish community occupying the mouth of hypothetical stage D, ecologically similar to that now found at the points 3. This is represented by the three daces and the Johnny darter.

As the hypothetical stage D eroded its bed and became stage E, which is represented by County Line Creek, fish community 3 was succeeded by a fish community ecologically similar to the fish community now present at points 4. This is ecologically represented by the three daces, the Johnny darter and the young of the common sucker. The fish communities designated as 1, 2, and 3 had meanwhile moved inland and were arranged in the order which their ecological constitution required.

The continuation of the process resulted in displacing a fish community ecologically similar to the fish community 4 by a fish community ecologically similar to the present fish community 5. This is represented in the lower waters of Bull Creek by the blunt-nosed minnow and the little pickerel. Before the destruction of its lower course, Pettibone Creek should have contained this community and represented stage F.

Ecological succession is one of the few biological fields in which prediction is possible. We may carry this discussion a little further. We have noted that the developing streams continue to erode their beds, grow larger, and bring down the surface of the land. These processes have not stopped in Bull Creek; it will become larger, contain a larger volume of water at the locality 5, and the fish community of locality 5 will be succeeded by a fish community ecologically similar to that now at locality 6. It is ecologically represented by the sunfish and bass present. This stage has been designated as hypothetical stage G in the diagram. With a further continuation of the process, the fish community of stage G, locality 6, will be succeeded by a fish community ecologically similar to that now found at the locality 7 (Bull Creek-Dead River. Stage H). The fish are shown in column 7, Table I.

(*b*) *Method and Principle.*

The discussion above is intended to illustrate a method of analysis as well as to illustrate the principles involved. In order to make both clear, more fish communities have been noted than otherwise would have been justified. It is important to recognize how much of the reconstruction is method, and how much is biological principle.

1. *The Biological Principle.*—The biological principle involved is one well recognized, but lacking in detailed and definite experimental confirmation. It may be stated as follows: Ecologically comparable animals living under similar conditions possess certain similarities of physiology, behavior, habits and mode of life.

Indirect evidence for this seems adequate for the purposes of this paper, but we have as yet been unable to conduct experimental studies in this line. The process of securing data to illustrate the principles involved here has not been small.

An obvious difficulty arises in securing language suitable for a brief expression of all that is included in physiology, behavior, habits and mode of life. The term *form* covers all matters of structure, size, proportion and in common usage, color also. As opposed to this and as covering all the physiological, and behavior characters just referred to, we will use the Latin word *mores*.[1] The *mores* of a *species* or *community of species* are not independent of the *form* or *forms:* the two are correlated, for every *mores*, we may expect to find some kind of structure, and for every form of structures, we must also expect to find some physiological differences. Our present methods may not detect these correlations between *form* and *mores* and the correlations may not be important, but the existence of such correlations seems to be a necessary assumption.

In such a series of streams as the north shore streams, it is a well-known fact that the conditions in the young streams are like those (near the headwaters) of the larger and older ones. It was for this reason that this series of streams was selected for the study. Since the same species of fish are present in like conditions, we have evidence for the uniformity of physiological make up. Since different species occupy different positions in the nicely graded series of complexes, we have an index of their comparative physiological make up which could not be easily detected by other means. We have located the animals in their environment, the first essential in study of their environmental relations.

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[1] *Mores:* behavior, customs. The word will be used as a collective noun exactly as form and forms are used in biology.

2. *Method.*—The method of analysis employed in this paper is based on physiography. The relation of physiography to ecology in this case is analogous to the relations of the microtome to anatomy, the electric needle to experimental morphology, and reagents to physiology. Ecology is in much the same state as was morphology when extensive papers and treatises on methods were necessary.

The object of this type of analysis and of locating the animal in its environment, is to determine the *physiological character of the animal as a whole.* The method of procedure is through the study of the complex conditions in which the organism is most nearly in physiological equilibrium. Some progress in the actual analysis of the organism can doubtless also be made by this method. Variation and modification of physiological make up may also be detected. Physiographic analysis is then a biological method. However, both the organism and the environment must be analyzed much further. In this further analysis the succession or the evolution of the environment is an important background and must form the basis for the selection of points of study, for comparison of results and organization in general.

Among geologists and some biologists, the presence of the same or similar taxonomic groups is often taken to indicate similarity of conditions. While ecologists must sometimes follow this method of reasoning, their method of procedure is in the main reversed. The *conditions* are studied and the presence or absence of a given set of organisms noted. In such a detailed set of conditions as these with which we are dealing, the old line of reasoning should not be followed. The modifiability of behavior speaks against it.[1] This is not a contribution to physiography. The physiography involved is well known to elementary students of that subject.

Physiographic analysis is a method like all other methods of science—to be improved, modified or rejected, according as it has

[1] *Asellus communis*, the common isopod, occurs in both ponds and streams. Mr. W. C. Allee has found that the behavior characters of the individuals inhabiting the streams and ponds are different. He has partially changed the stream *mores* to pond *mores* by keeping them in pond conditions and vice versa.

served its purpose or shows its defects. It is not, however, the only method of ecology, and it should not be employed alone, but accompanied by experimentation.

This same method may be employed in the study of the historical problems of biology, or to the study of evolution, but the results are not ecology because this method is employed. Nor is there any intimate dependence between the fundamental things in ecology so far as its progress as a science is concerned, and the divisions of biology known as evolution, morphology, or faunistic geography.

Physiographic analysis is only a part of a more general method of deducing succession and laying a foundation for comparisons. The general method of successional study has a probable significance which lies beyond the recognition of the physiological characters of organisms as a whole and the analysis of organisms as far as the method will permit.

(*c*) *The Significance of Succession.*

It will be noted that in the above statement of succession no reference to species is made. Species in the morphological sense can have only the most local significance in succession. Species inhabiting similar stages in the physiographic succession of streams, *i. e.*, similar conditions—will hardly be the same within a very small area. It appears that the idea of succession has been regarded as having little significance for this reason. A point of significance is the character of the *mores*. In the matter of their *mores* the fish communities of a stream in Europe, a stream in Japan, and a stream in the United States are probably similar if the streams are similar—a matter for experimental verification.

A science with only one method of classifying its data must constantly fall into error. Up to the present we have but one organized general system of classification for zoölogical materials, namely taxonomy. Taxonomy has been over-emphasized and carried into fields where it does not properly belong. It has given us, among other things, what has been called *composite natural history*. Composite natural history constitutes a large part of the natural history of today, especially as contained in general works on natural history. The development of a composite natural history generalization consists in discovering that certain habits are characteristic of a few known (as to habits) members of a given taxonomic group of animals, in striking a sort of general average of the known habits of the members in question and putting such an average into the form of a general statement to apply to all the group, known or unknown. Such generalizations have their place in didactics, but they are the most inaccurate phase of zoölogy. Though often used as a basis for generalization, generalizations based upon them have a questionable value.

The crucial question before us is, then, Can we, on the basis of ecological succession or other natural basis, classify and compare animal *mores* and make generalizations on the basis of such classification and comparison? If investigation answers in the affirmative, we may organize a general system for the classification of zoölogical materials which shall be sufficiently independent of other methods and systems of classification to serve as a means of throwing additional light on the generalizations of other zoölogical fields, such as physiology, heredity, or evolution. Indeed, plant ecologists have progressed far with such a classification (Warming, '95; Cowles, '01, and many others). Their results are now recognized as of much importance by botanists generally. The logic and method back of their classification is the same as ours. The differences lie in the fact that differences in physiological make up in plants is usually indicated by vegetative *form* while differences in physiological make up in animals is indicated by differences in *mores*.

Ecological succession has not as yet been studied experimentally. Experimental studies, if properly conducted, will answer the question of the significance of all the propositions here presented. Such experimentation should be conducted with reference to an *analyzed environment* and should consist of the comparison of the *mores* of animals of different and of similar environments.

V. SUMMARY.

1. Fishes have definite habitat preferences which cause them to be definitely arranged in streams which have a graded series of conditions from mouth to source.

2. Beginning at the sources of the streams of the developmental series considered, we find the same species represented in essentially the same order in all the streams, in so far as the series of conditions is present. The only species in the youngest stream is the same as the species nearest the sources of the larger streams.

3. Migration of conditions for breeding is an important cause of fish migration, but fish reactions outside the breeding season may be often more important than movement of conditions necessary for breeding over the route of migration. Migration may even be due to reactions to a single factor.

4. Fish entering a stream will take a position in the stream suited to their ecological constitution without regard to the time and mode of origin of these conditions.

5. There is a succession of ecological types over a given point. Ecological succession is based on similar *mores* (physiology, behavior, habits and mode of life) of fish communities as a whole or comparable species of communities.

6. Physiographic analysis locates the animal in its environment and is but a method of studying the organism as a whole and a basis for proceeding to its analysis.[1]

VI. Acknowledgments and Bibliography.

The identification of the fish discussed in this paper are by Dr. S. E. Meek and Mr. S. F. Hildebrand, and of the Mollusca by Mr. F. C. Baker. Some of the seining was done in cooperation with Mr. Hildebrand. Without the assistance of these gentlemen this paper would not have been written. I am indebted to Professor F. R. Lillie and Mr. Ellis L. Michael for criticising the manuscript.

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EFFECTS PRODUCED BY CUTTING PARAMECIUM CELLS.

GARY N. CALKINS.

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In a previous paper on *Uronychia* I have shown that the power of regeneration in a hypotrichous ciliate is a factor of cell age, increasing as the cell grows older after division, until a maximum power is attained just prior to cell division. In the present paper I desire to describe the results obtained by similar methods of experimentation on a much more difficult subject, the holotrichous ciliate *Paramecium*. Here in forms from ordinary cultures, the power of regeneration is very poorly developed and the nuclear apparatus is more concentrated. Similar experiments on *Paramecium* have often been attempted, but the difficulties in technique are so great that, with the exception of Balbiani's beautiful work in 1893, little or no extensive work has been done. The endeavor to ascertain what happens in a cell in which the power of regeneration is slight and when the physico-chemical equilibrium is suddenly disturbed is a problem well worth the patience and repeated failures necessary in getting the small percentage of success, and the results obtained at intervals during the last three years are here brought together for the first time. In a subsequent paper I shall publish my results with another race of *Paramecium caudatum* on regeneration in relation to cell division.

Material and Method.

Of the thousands of *Paramecium* that I have succeeded in cutting with a scalpel as in the *Uronychia* work, only about two hundred, of which one hundred and fifty approximately are here described, have given complete records. The cell to be operated is placed on a glass slide with a drop of water of minimum size and the cell is cut with a fine scalpel under a binocular microscope. It is a comparatively simple matter to actually cut the cell in two parts but if the knife passes through the macronucleus or its membrane, the fragments are useless for the nucleus is never retained and both parts quickly collapse. The results show a marvellously delicate coördination of the parts of the cell, a very slight injury, as the following records will show, throwing the physiological mechanism out of gear.

Immediately after cutting a cell the peristome depression fills out, leaving the cell periphery perfectly smooth; the mouth cavity, however, does not disappear but persists as a miniature cave in the protoplasm. In from fifteen minutes to half an hour after the operation the part remaining of the old peristome reappears, movements of translation and rotation are continued as before, but in most cases the cell is truncated and the cut surface appears as clean cut and smooth as though a wax model of *Paramecium* had been cut with a sharp knife. If the cut cell lived for four hours after the operation it was recorded as a successful experiment and the results here described were made on cells that invariably recovered from the shock of the operation and lived at least four hours afterwards. Fragments living after this four hour test were kept isolated in small watch glass culture dishes in a moist chamber. The culture fluid was 24-hour-old hay tea made by boiling a small quantity of hay in tap water. The division rate of normal *Paramecium* kept under similar conditions varied from one to three divisions per day according to the race under observation.

In the case of *Uronychia* both fragments of the cell resulting from a single cut, continue to live for at least 24 hours, and frequently such fragments continue to live for three or four days without regeneration. With *Paramecium*, on the other hand, the smaller fragment almost always collapses. One or two exceptional cases of continued activity may be mentioned here. One, a giant form, was cut through the mouth at 11.15 A.M. At 4 P.M. both fragments were alive, the posterior fragment swimming actively with the truncate end in advance, the anterior fragment with the truncate end behind. On the following day the smaller posterior fragment was dead, the other, anterior, lived for 72 hours when it was killed (no. 6). A second case, cut in the same place, resulted in two fragments both of which were alive after six hours. On the following day the smaller one was dead but the other lived and gave rise to two types of cells, one much smaller than the other and with a blunt posterior end, while the other had the characteristic pointed end of *Paramecium caudatum.* In still another case the giant form was cut posterior to the mouth, the posterior fragment being about one half the size of the anterior part. Both parts were alive after six hours but both were dead on the following day.

In all of these cases the organisms were treated for from fifteen minutes to one half an hour with dilute neutral red, granules in the cell being deeply stained at the time of cutting. What effect the neutral red has upon the consistency of the protoplasm I do not know, but certainly there was a marked difference in the resistance to collapse after treating with this dye. In only two other cases have I succeeded in keeping both fragments alive after the operation on normal forms; in one case the smaller fragment lived only ten minutes after the operation; in the other case (Table II., no. 63) the original cell was treated with nuclein before cutting, and both parts lived 24 hours. In the majority of cases the smaller fragment collapses immediately after removal of the knife.

Several different races of *Paramecium* have been used, but giant forms on the whole have been selected because of the greater ease of cutting. The smaller races and races of *Paramecium aurelia* do not allow sufficient play for the knife edge and too great a zone is crushed by the operation. One race of giant forms was obtained from brackish water at Roscoff, France, for which I am indebted to Mlle. Lipska. The other races have been obtained from time to time from wild cultures in the laboratory at Columbia University. As there is little or no difference in the percentage of successful results in the different races I shall not particularize but will deal with them all as of one race. Up to the present in these experiments, no systematic effort has been made to study the regenerative power at different periods between division phases, as in the studies on *Uronychia*. The percentage of cases of regeneration was so small that such a study seemed valueless, but with a race now under experimentation more satisfactory results are expected. Of the seven cases of regeneration obtained in the one hundred and forty-nine cases here recorded, five were cells that were cut while either dividing or conjugating, results indicating that not only division age but racial age is also a potent factor in regeneration. Further work on this phase of the subject is now under way and will undoubtedly yield interesting results.

In the following tables the experiments are grouped according to the region of the cell originally cut. The cell is by no means homogeneous and the different portions are not equipotent. If we imagine the *Paramecium* long diameter to be divided into four equal parts by three cuts, the central one would pass through the plane of division of the cell, the upper one would divide the anterior half into two dissimilar quarters, the lower one would divide the posterior half of the *Paramecium* into dissimilar quarters. In the tables, the zones indicating these several quarters are numbered from one to four, 1 being the most anterior, 2, the next anterior, 3 the posterior central zone and 4 the terminal posterior zone (text figure 1). The most important organs of the cell are contained in zones 2 and 3, zone 2 containing the macro- and micronucleus, and zone 3 containing the mouth. Zones 1 and 4 do not contain any of the more important organs, and yet, as the sequel shows, these parts if injured are rarely replaced and their absence has a remarkable effect on further activities of the cell.

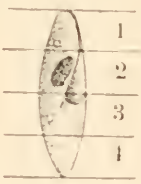

FIG. 1.

TABLE I.

Paramecium caudatum CUT IN ZONE I.

	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks
No. 1	Active. No regeneration.	Divided. One truncate, one normal.	Normal divided, other not regenerated.	Killed.
No. 2	No regeneration.	Dead. No regeneration		Conjugating when cut.
No. 3	Two normal cells. One small.	Small one dead, other dividing.		Dividing when cut.
No. 4	No regeneration.	No regeneration.	No regeneration. Killed.	Fig. 2, Plate I.
No. 5	No regeneration.	No regeneration. Control divided.	No regeneration.	
No. 6	Cut end ciliated.	Divided twice, four normal cells.		
No. 7	Dead.			
No. 8	Dead.			
No. 9	Dead.			
No. 10	Dead.			
No. 11	Dead.			Fig. 1, Plate I.
No. 12	Dead.			
No. 13	No regeneration.	Dead.		
No. 14	No regeneration.	Dead.		
No. 15	Dead.			
No. 16	Small, sluggish, deformed.	Dead.		
No. 17	No regeneration.	Dead.		
No. 18	Dead.			
No. 19	Dead.			
No. 20	No regeneration.	Divided. One small. Rounded ends.	Both alive and divided (see description).	

TABLE II.

Paramecium caudatum CUT IN ZONE II.

Expt.	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 1	Unhappy. No regeneration.	Sluggish, no regeneration. Killed.		No nucleus in cell.
No. 2	No regeneration.	No regeneration.	No regeneration.	Conjugating when cut. See p. 49.
No. 3	Cell divided in original plane	Both cells divided once	No regener. Same as before	Cell in conjugation when cut. Fig. 3, Plate I.
No. 4	Normal posterior. Abnormal anterior divided in original plane.	Both cells normal. Regeneration perfect.	Both dead.	
No. 5	Dead. No regeneration.			
No. 6	Monster. 2 mouths.	Monster.	Monster.	Killed 96 hours. See p. 53. Fig. 18, Plate III.
No. 7	Dividing in orig. center. Killed.			Fig. 4, Plate I.
No. 8	Dividing.	Both dead.		
No. 9	Divided in orig. center.	No regeneration. Killed		Fig. 5, Plate I. See p. 49.
No. 10	No regeneration.	Dead.		
No. 11	Normal.	Normal.		Dividing when cut. Fig. 10, Plate I.
No. 12	Dead.			
No. 13	Dead.			
No. 14	No regeneration.	Left in culture. 10 days later both large and small forms in culture dish. See p. 51.		
No. 15	Rounded anteriorly. No growth.	Dead.		Neutral red.
No. 16	Dead.			
No. 17	Dead.			
No. 18	Dead.			Neutral red one half hour.
No. 19	Dead.			
No. 20	Dead.			
No. 21	No regeneration.	Divided in original plane.	Both dead.	Fig. 6, Plate I.
No. 22	Divided in original plane.	No regeneration.	Monster.	See p. 56. Fig. 16, Plate II.
No. 23	Dead.			
No. 24	Dead.			

TABLE II.—*Continued.*

Expt.	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 25	Dividing in original plane.	No regeneration. Monster.	Monster.	See p. 57. Fig. 20, Plate III.
No. 26	Dead.			
No. 27	Dead.			
No. 28	Dead.			
No. 29	Dividing in original plane.	Dead.		Fig. 8, Plate I.
No. 30	Dead.			
No. 31	Dead.			
No. 32	Dead.			
No. 33	Dead.			
No. 34	Dead.			
No. 35	Divided in original plane.	Monster.	Monster.	See p. 54.
No. 36	Dead.			
No. 37	Dead.			
No. 38	Dead.			
No. 39	Divided in original plane.	Monster.	Monster.	See p. 54.
No. 40	Divided in original plane.	Monster.	Monster.	See p. 55. Fig. 17, Plate II.
No. 41	Dead.			
No. 42	Dead.			
No. 43	Dead.			
No. 44	Dead.			
No. 45	Dead.			
No. 46	Dead.			
No. 47	Dead.			
No. 48	Dead.			
No. 49	Alive. No regeneration.	Dead.		
No. 50	Dead.			
No. 51	Divided in original plane.	Monster.	Monster.	See p. 54. Fig. 14, Plate II.
No. 52	Dead.			
No. 53	Dead.			
No. 54	Large. No regeneration.	Larger. No regeneration.	Very large. No regeneration.	Divided in 96 hours. See p. 50. Fig. 7, Plate I.

TABLE II.—*Continued.*

Expt.	Condition after 24 Hours	Condition after 48 Hours	Condition after 72 Hours	Remarks.
No. 55	Dead.			
No. 56	Dead.			
No. 57	Lively. No regeneration.	Dead.		
No. 58	No regeneration.	Dead.		
No. 60	Active. No regeneration.	Dead.		
No. 61	Dead.			
No. 62	Very weak.	Dead.		
No. 63	Both fragments alive and active.	Dead.		
No. 64	No regeneration.	Dead.		
No. 65	Dead.			
No. 66	Dead.			
No. 67	No regeneration.	No regeneration.	Dead.	
No. 68	Larger. No regeneration.	Larger. No regeneration.	Monster.	See p. 54.

TABLE III.

Paramecium CUT IN ZONE III.

	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 1	No regeneration.	No regeneration.	No regeneration.	See p. 51.
No. 2	Both cells truncate. No regeneration.	Both dead.		Conjugating when cut.
No. 3	No regeneration.	No regeneration.	No regeneration.	Ex-conjugant. Killed 96 hours.
No. 4	No regeneration.	No regeneration.	No regeneration.	Killed 96 hours ; ex-conjugant. Fig. 12, Plate I.
No. 5	Dividing in original plane.	Dead.		
No. 6	No regeneration.	Monster. 2 mouths.	Monster. Killed.	See p. 54. Fig. 19, Plate III.
No. 7	Large. No regeneration.	Larger. No regeneration.	Very large. No regeneration.	Killed 96 hours. See p. 52.
No. 8	Dead. Cells united.			Conjugating when cut.
No. 9	Two truncated cells.	No regeneration.	No regeneration.	Both dead 96 hours. No regeneration. Conjugating when cut.
No. 10	No regeneration.	No regeneration.	No regeneration.	Dead 96 hours. No regeneration.
No. 11	No regeneration.	Monster. 2 mouths.	Monster.	See p. 54.
No. 12	No regeneration.	Very large, no regeneration.	Divided, one normal, other abnormal.	Killed 96 hours.
No. 13	No regeneration.	No regeneration.	No regeneration.	Killed 96 hours. See p. 51.
No. 14	Dead.			
No. 15	Dead.			
No. 16	Dead.			
No. 17	Divided in original plane.	Very large.	Monster.	See p. 55. Fig. 15, Plate II.
No. 18	Dead.			
No. 19	Huge. No regeneration.	Divided in original plane.	Abnormal, cell very large.	See p. 52. Fig. 9, Plate I.
No. 20	Dead.			
No. 21	Dead.			
No. 22	No regeneration.	Dead.		
No. 23	No regeneration.	No regeneration.	Monster.	See p. 54. Fig. 13, Plate II.
No. 24	Divided, both dead.			
No. 25	Dead.			
No. 26	Dead.			

TABLE III.—*Continued.*

	Condition after 24 Hours	Condition after 48 Hours	Condition after 72 Hours	Remarks
No. 27	Dead.			
No. 28	Dead.			
No. 29	Dead.			
No. 30	Dead.			
No. 31	Dead.			
No. 32	Dead.			
No. 33	Dead.			
No. 34	No regeneration.	No regeneration	Dead	
No. 35	Dead.			
No. 36	Dead.			
No. 37	Dead.			
No. 38	No regeneration.	Dead.		
No. 39	No regeneration.	Dead.		
No. 40	Dead.			
No. 41	No regeneration.	Monster.	Monster.	See p. 54.

TABLE IV.

Paramecium caudatum CUT IN ZONE 4.

	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 1	Regeneration perfect.	Normal. Divided.	One weak, other cut.	Dead.
No. 2	No regeneration.	Dead.		
No. 3	Regeneration perfect. Cells very small.	Normal.	Normal.	Conjugating when cut. See p. 53.
No. 4	Both dead.			Conjugating when cut.
No. 5	Slightly truncate, crystals in anterior end.	Same.	Dead.	Cut during division. Fig. 11, Plate I.
No. 6	Dead.			
No. 7	Dividing but cannot separate.	Separated but dead.		
No. 8	Dead.			
No. 9	Dead.			
No. 10	Dead.			
No. 11	Dead.			
No. 12	No regeneration.	No regeneration.	No regeneration.	Dead after 120 hours.
No. 13	No regeneration.	Dead.		
No. 14	Dead.			
No. 15	Divided, one normal, other truncate.	Both dead.		
No. 16	No regeneration.	No regeneration.	No regeneration.	By fifth day divided twice, all normal.
No. 17	Dead.			
No. 18	Dead.			
No. 19	Dead.			
No. 20	Dead.			

Table V.

Summary of Tables I.–IV.

Zone of Cut.	No. of Cases.	Alive after 24 Hours.		Alive after 48 Hours.		Alive after 72 Hours.		Monsters.		Regeneration.	
		No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
1	20	11	55.5	4	20.	2	10.	0	0	2	10.
2	68	29	42.6	14	20.6	11	16.2	8	11.7	3	4.4
3	41	20	48.8	15	36.6	14	34.1	5	12.2	0	0
4	20	8	40.	4	20.	4	20.	0	0	2	10.
Total	149	68	45.6	37	25	31	20.8	13	8.7	7	4.7

2. Analysis of Tables and Experiments.

1. *Analysis of Table I.*

In the experiments included in this table the cells were cut in zone 1 or through the anterior quarter of the cell, a part containing only the anterior terminus of the peristome but none of the important cell organs.

Of the 20 cases recorded as having lived at least four hours after the operation, some (9) died within 24 hours; others (5) died without regeneration or division within 48 hours; others (3) lived for 72 hours or longer without regeneration; others (2) ultimately gave rise to normal descendants, and one divided within 24 hours giving rise to normal cells.

Number 11 is a good example of the 9 cases of death within 24 hours. Here a clean cut resulted in an anterior small fragment which instantly collapsed, and a larger rounded fragment in which the peristome was obliterated. Within half an hour the peristome reappeared exactly as it was before except for the small part removed (Fig. 1). No rearrangement of the protoplasmic material occurred and the old form was perfect except for the missing part. So far as external appearances were concerned the cell should have lived.

Of the five specimens that died within the next 24 hours, number 16 is a good example. Here no effort was made to regenerate and at the end of 24 hours the fragment was small and sluggish with the remainder of the peristome perfect. The cell died before the end of the next 24 hours.

Number 4 is a good example of a fragment that lived for 73 hours without regeneration or division. The cut surface had developed cilia and regeneration had progressed to that extent but no further (Fig. 2).

The two cases of regeneration were 3 and 6, only one of which was a vegetative form, the former being cut while dividing the latter during a vegetative stage. Number 3 was first cut in the plane of division separating the two cells; one of these was then cut again in the first zone. On the following day both cells were normal *P. caudatum*, but the cell that had been cut was much smaller than the sister cell. The cut one died on the third day.

The vegetative form that regenerated showed after 24 hours an anterior end somewhat blunt but rounded and ciliated, and on the following day it had divided twice, giving rise to four cells which could not be distinguished from normal cells. The double division indicates that the original cell was about ready to divide at the time of cutting and that removal of the anterior part had no effect on the subsequent divisions.

Analysis of Table II.

The mortality of *Paramecium* cut through zone 2 is considerably greater than that for zone 1. One chief reason for this is probably the fact of the injury or destruction of the macronucleus which usually occupies this region. Of the 68 cases 47.4 per cent. died before the end of the first 24 hours; 79.4 per cent. were dead before the end of 48 hours; but if the fragments lived through this period there was a good chance of continued life for some days, at 72 hours for example there were only 83.8 per cent. dead, only a slight increase over the mortality at the end of 48 hours. The majority of these or 8 out of 11 were monsters while 3 out of the 68 regenerated into perfect cells.

Of those that lived 48 hours or more after cutting, some (5) failed to divide or else formed monsters; others divided in the plane of the original center of the cell giving rise to (*a*) cells with reduced vitality which soon died; (*b*) one normal cell and one abnormal which died; (*c*) one normal cell and one abnormal which divided more than once; (*d*) one normal and one abnormal cell which formed a monster, and (*e*) two normal cells.

Of the four cases living 48 hours or more in which no division occurred, the history was varied; the experiments were numbers

1, 2, 67 and 68 of Table II. Number 1 was killed after 48 hours and found to have only a few nuclear fragments. Number 2 was killed at the end of 96 hours, and, although cut during the process of conjugation, was found to have a normal macronucleus. Number 67 was dead at the end of 72 hours, no regeneration occurred but the cell was very lively and examination showed a normal macronucleus. Number 68 was a vegetative form which recovered perfectly from the shock of the operation and lived without regeneration or division for a period of three days.

Of the cases in which the cut fragment divided in the original central plane of the cell, the dividing fragment was killed in two cases for the determination of the nuclear conditions (*a*); both daughter cells died in 3 cases (*b*); an abnormal cell was formed which divided three times in one case (*c*); monsters were formed in 7 cases (*d*); and regeneration occurred in 3 cases.

(*a*) The dividing fragment was killed either during division or immediately afterwards to determine the conditions of the nuclei in experiments 7 and 9. Number 7 is shown in Fig. 4. The posterior cell (4, *c*) is apparently normal, the micronucleus is dividing normally but the macronucleus is somewhat distorted. Number 9 was evidently an ex-conjugant when operated and dividing for the second time. It was killed 24 hours after division; the abnormal cell had one of the four new macronuclei and several of the degenerating fragments of the old macronucleus; the other cell was a normal exconjugant (Fig. 5, Plate I.).

(*b*) Both daughter cells died without regeneration in experiments 8, 21 and 29. Number 21 is a good example of these cases. Here the cell was cut as shown in Fig. 6, *a*. On the following day there was a small tentacle-like appendage on the cut surface (6, *b*). On the second day the fragment had divided through the original center of the cell into dissimilar daughter cells (6, *c*), one of which was normal in shape, but with a collection of black granules at the anterior end (6, *c*); the other cell was small and as sharply truncated as when cut (6, *d*). On the third day both were dead.

Number 29 was similar in history but the abnormal fragment formed by division of the cut cell was extremely minute, being little more than a small sphere with a nucleus and a contractile vacuole. Its small size was due to the proximity of the cutting plane to that of the normal division plane of the cell. It died on the second day after cutting (Plate I., Fig. 8, *a*, *b*, *c*, *d*).

(*c*) *Division of the Abnormal Cell.*—In one experiment of this series (no. 54) the abnormal cell, resulting from the division of the original cut fragment, divided three times. The vegetative cell was cut as shown in Fig. 7, *a*. After 24 hours the fragment was lively and complete as far as the plane of the cut which was sharply marked (Fig. 7, *b*). After 48 hours it had grown much larger and was particularly broad at the anterior or truncate end. After 96 hours it had divided into an abnormal truncate cell (7, *c*, and 7, *d*) and a normal cell (7, *e*). On the sixth day this abnormal cell had grown in size but retained the original truncate anterior end (7, *f*) while the normal cell had divided to form two normal cells of which one was cut (see Table III., no. 38) and the other lost in cutting. On the seventh and eighth days the abnormal cell had grown very large again and on the ninth day divided to form two small dissimilar cells (7, *g*, *h*), each, however, with only one vacuole. On the twelfth day each of these divided once more giving rise to four small abnormal cells (7, *i*, *j*, *k*, *l*), one of these (*l*) dying on the same day, two of the others (7, *m*, *n*) dying on the seventeenth day and the fourth (*o*) on the eighteenth day.

This experiment resulted in two races of cells, one of which (from the posterior end) were normal, the other abnormal. The part lost was never regenerated and the abnormality was finally transmitted to the posterior end of the original fragment.

(*d*) *Monster Forms.*—These will be considered together with those of Table III. (see page 53).

(*e*) The three cases of regeneration were nos. 4, 11 and 14. Of these no. 4 was cut during division of a vegetative cell. Within half an hour after the operation the division was completed, one perfect cell and a small imperfect cell resulting. In 24 hours both were living and both were of normal shape but one was much smaller than the other. At the end of 48 hours the small one had grown to a normal cell both in size and shape, but on the third day it was dead.

No. 11 was also a dividing cell when cut through zone 2. On the following day the larger fragment appeared entirely normal save for an accumulation of crystals at the anterior end (Fig. 10, *a*, *b*); the smaller cell was truncate. On the second day both were normal.

No. 14 was a large vegetative cell which had been treated with neutral red prior to cutting. The smaller fragment, contrary to the usual history, remained alive for six hours but was dead the next day. The larger fragment did not regenerate within 48 hours. The watch glass containing the fragment was not examined again for a period of eleven days when it was found to contain 12 large giant forms and eight small forms normal save for size.

Analysis of Table III.

The cells cut in zone III. gave a higher percentage of living fragments than those cut in zone II. 48.8 per cent. continued to live after 24 hours as against 42.6 per cent.; while 36.6 per cent. continued to live after 48 hours as against 20.6 per cent. The percentage of monsters in both zones was about the same, viz., 12.2 per cent. and 13.2 per cent. but there was not a single case of regeneration. Of those that lived but did not regenerate, some (3) were cells just after conjugation; others (2) were conjugating when cut, while the remainder were large vegetative forms. The reactions for the most part were similar to those of cells cut in zone II., the conjugating forms, or those just out of conjugation, giving the only novel results. These were nos. 1, 2, 3, 4, 8 and 9 of Table III. In nos. 1, 3 and 4 the cells were ex-conjugants when cut, the fragments being killed after 96 hours for the determination of the nuclear condition. Fig. 12 shows the structure of nos. 1, 3 and 4 at this period, the characteristic fragmented nucleus showing a terminal stage of conjugation. It is possible that these cells would have continued to live and to divide had they had a longer time, for they were active and normal in appearance when killed. It was quite otherwise, however, with cells cut during conjugation (nos. 2, 8 and 9). In all cases the fragments died within four days without any attempt at regeneration. In one case (no. 8) the cells were still united but dead after 24 hours; in another (no. 9) both lived for several days without regenerating or dividing; in another case (no. 2) they separated and lived for 48 hours when both died.

Of the vegetative forms the most interesting cases were nos. 7 and 19, the former typical of the majority of cases, the latter unusual. No. 7 was a small form cut just below the mouth. It grew very large but did not regenerate nor divide in the four days of observation, and was finally killed. The nucleus was normal although many fragments of macronuclear material indicated that the original cell was a recent ex-conjugant and was in about the fourth generation when cut.

No. 19 was more interesting. It was cut just below the mouth as in the preceding case. In 24 hours it had grown very large and had divided through the original center of the cell, forming one normal and one truncated cell (Fig. 9, Plate I.). The normal cell divided again before the third day forming two perfectly normal paramecia which were then discarded. The truncated cell grew very large before the third day and divided on the fourth day into one small abnormal cell (Fig. 9, *b*, *c*, *e*) and one larger normal cell (Fig. 9, *d*). On the fifth day the larger, normal form had divided while the small truncated one had developed a sharp posterior end and was almost normal in appearance; finally on the tenth day, all were living and all normal save for a noticeable difference in size (Fig. 9, *g*, *h*, *i*, *j*). Here, therefore, there was a gradual return, through four or more generations to the normal form, although the single cell failed to regenerate.

Analysis of Table IV.

Cells cut in the extreme posterior end were seriously damaged although the abnormalities were not so marked as in the other experiments and 10 per cent. of them regenerated. Only 40 per cent. were alive after 24 hours and only 20 per cent. after 48 hours showing that, although not much mutilated, vitality was nevertheless badly affected by the operation. There were no monsters, although in one case (no. 7) the cell divided 24 hours after cutting but the daughter cells had great difficulty in separating, remaining attached by a narrow isthmus of protoplasm for one full day and finally separating. Within a few hours after separation, however, both cells died. This remarkable effect was produced by cutting off only the most posterior tip of the original cell. It seems hardly credible that so marked an effect can be produced by such a minor mutilation and it suggests the possibility pointed out by McClendon, of different electrical states at different portions of the cell.

Again, in no. 12 the fragment lived for five days without division or regeneration and finally died. Peristome and mouth were normal but the loss of the posterior end appeared to be fatal.

No. 15 divided 24 hours after cutting forming one truncated posterior cell and a normal anterior cell. The physiological balance, however, was again disturbed for both cells died on the following day.

No. 16 was not so seriously affected by the operation; the cut fragment grew larger each day up to 72 hours when it divided into one truncated and one normal cell, the latter dividing again on the fifth day, the former dividing on the sixth, forming practically normal cells.

Of the forms that regenerated (nos. 1 and 3) both were conjugating when cut. In no. 1 both cells were cut, separation followed normally and each cell had filled out normally by the following day. One, however, grew more and more feeble until it finally died. The other was entirely normal and was used for experiment 21. In no. 3 only one cell of the pair was cut, the other remaining uninjured. After 24 hours both were perfect in form, although one was perceptibly smaller than the other. Both died on the sixth day.

Monster Forms.—Of the 13 monsters only one resulted from a specimen that was cut during division, all of the others came from fragments of vegetative cells. Eight of the thirteen were cells that had been cut in zone II., the other five in zone III. In all cases the power to divide was limited to the nucleus, the cell body apparently being incapable of division.

In no. 6 (Table II.) the cell was dividing when cut through the anterior half of the posterior cell (Plate III., Fig. 18). The fragment lived for three days when it was killed for nuclear structures. Twenty-four hours after cutting the posterior part developed a new peristome and mouth on the cut surface, but the original division was never completed. When killed the cell had one large nucleus which had grown during the three days. The other nucleus had apparently been cut away.

No. 6 (Table III.) was a similar monster, but was derived from a normal vegetative cell cut in zone III. After three days the fragment had attempted to divide; a constriction was present but the posterior cell was only a swelling with a mouth opening and no peristome (Plate III., Fig. 19, *a*, *b*, *c*).

In the remaining eleven cases of monster formation many degrees of malformation resulted from the operation. Of these the least monstrous was no. 23 (Table III.). The original cell was cut as shown in Fig. 13, Plate II. There was no sign of regeneration during the next three days but the cell grew larger and finally attempted to divide (Fig. 13, *b*, *c*). One of the cells was normal in shape and size; the other, posterior, was smaller and truncated. The two remained attached for a period of three days swimming about actively, bending and twisting with the various cilia in action, until they finally died, undivided, nine days after the operation. The nuclear apparatus was not determined. In this case two complete peristomes and mouths were developed and the cells were attached by only a delicate strand of protoplasm. The general result was similar to that of experiment no. 7 (Table IV.) in which the daughter cells remained attached for 24 hours and finally separated, dying shortly after the separation (see page 52).

Other monsters formed without division of the cut cell were nos. 35, 39, 40, 51 and 68 of Table II., and nos. 11 and 41 of Table III. Nos. 35 and 39 were killed after 4 and 5 days respectively, while nos. 68, 11 and 41 died in from 4 to 8 days. The other two, nos. 40 and 51, lived for 20 and 17 days respectively, and developed into relatively huge protoplasmic masses.

The remaining three monsters, nos. 22 and 25 of Table II., and no. 17 of Table III., all came from an original form that divided after the operation into one normal and one abnormal cell, the monster in all cases coming from the abnormal cell.

The history of only the most interesting of these monsters need be given, all matters of importance in the other cases being included in the history of these.

The simplest case is no. 51 which lived, after cutting, for 17 days undergoing two attempts to divide in that time. The cell was cut as shown in Fig. 14, Plate II., in zone II., and the fragment was very lively for 72 hours growing enormously during that period (Fig. 14, *b*). Between the third and the fifth day it divided giving rise to a monster as shown in Fig. 14, *c*. The mass had two peristomes and two mouths placed as shown in the figure, and continued to grow in size changing form from day to day and adding a new mouth on the sixteenth day. It died on the seventeenth day. The nuclear apparatus had disintegrated with death of the mass and could not be determined. This case gave the largest mass of protoplasm obtained, with the fewest cell organs, only one contractile vacuole being observed and with only two mouths for most of the time. The power to grow was intact and the mass great enough for six cells.

No. 17 (Table III.) is another simple case of monster formation which differed from the foregoing in appearing after the first division subsequent to the operation. The cell was cut in zone III. as shown in Fig. 15, *a*. On the following day the fragment had divided into an abnormal and a normal cell (*b*, *c*, *d*). On the second day the normal cell divided while the abnormal one remained as before save for growth. On the third day the normal cell divided once again into two normal cells, and the abnormal one attempted to divide but formed a monster with a small anterior and a larger posterior portion. There were two mouths, two peristomes and two contractile vacuoles. On the fourth day the monster had changed somewhat in axial relations so that the forms of two paramecia could be made out. The mass however was weaker than on the preceding day and died on the following (fifth) day. A preparation showed the presence of two macronuclei and two micronuclei in their appropriate positions (Fig. 15, *e*).

Experiment 40 (Table II.) gave one of the most interesting of the monsters. The cell was cut in zone II. on the fourth of February. Forty-eight hours later it had attempted to divide in the original center of the cell but formed a monster with a small anterior cell and a full size posterior cell (Fig. 17, *a* and *b*). Two complete peristomes with mouths were present and two contractile vacuoles. These conditions were retained without further morphological change save growth in size until February 10, or the sixth day, when a drop of nuclein was added to the ten drops of hay infusion medium. On the seventh day the monster had grown enormously (Fig. 17, *c*); on the ninth day it gave rise to a small free cell which was an exact miniature copy of the original cut fragment (17, *e*). This cell arose from the upper end of the monster as shown in 17, *d*. On the tenth day the isolated fragment was killed for the determination of the nuclear apparatus and was found to have a normal full size macronucleus. On this day also, the monster divided at the point *x* forming two monsters, one having three mouths (17, *g*) the other with only two (17, *f*), but the latter at the time of division, was also dividing to form a small terminal deformed cell. Thus two divisions were going on at the same time in the protoplasmic mass. The small deformed cell was detached from the parent monster on the same day and appeared as a minute reversed duplicate of the original fragment (Fig. 17, *h*). This, like the first detached fragment, had a single contractile vacuole. On the eleventh day the small fragment was still alive and active but had not regenerated nor grown in size while the two monsters had grown to look more like paramecia fused (17, *i* and *j*). On the twelfth day the small fragment was dead while the others remained as before. On the thirteenth day the smaller monster had grown much weaker and was killed on the eighteenth of February or the fourteenth day (17, *k*). The remaining monster was very plastic changing shape from day to day until on the twenty-fourth of February appearing very weak, it was killed, twenty days after the original operation during which time no more than three mouths were formed in this part of the divided monster. The original fragment thus developed seven mouths indicating at least seven attempts to divide while the monster divided once to form two monsters. Preparations (17, *k* and *l*) show relatively enormous nuclear masses.

One interesting feature of this experiment was the formation of free-living but abnormal fragments separated off from the protoplasmic mass. In experiments nos. 22 and 25 (Table II.) the monsters gave rise to similar cells either attached (22) or free (25), but in every case these offshoots were normal in structure although abnormal and weak in function.

In No. 22 the cell was cut in zone II. as shown in Fig. 16, *a*, leaving the lower part of the peristome, the nucleus and the mouth intact. Twenty-four hours later the truncate cell divided in the plane of the original center into a small truncated anterior fragment (*b*, *c*, Fig. 16), and a normal cell (16, *d*). The abnormal fragment had one contractile vacuole, the normal cell two. Seventy-two hours after the original cut the normal cell (*d*) divided into two perfectly normal paramecia, which divided again on the following day into normal forms, thus establishing the entirely normal condition of this product of the original truncate fragment. The abnormal fragment (*c*) neither regenerated nor divided for the first five days after the operation, although it grew considerably in size (16, *e*). On this fifth day, however, it attempted to divide, but cytoplasmic division failed and a monster with two mouths, two contractile vacuoles and two blunt ends was formed (16, *h*). The power of division seemed to be again restored for on the sixth day the mass attempted a second division, and this time a perfect *Paramecium* was formed, but, as in experiment no. 7, Table IV., it could not separate from the parent mass and remained attached by a delicate connection throughout life of the monster (Fig. 16, *i*, *j*). On the seventh, eighth, and ninth days the monster gradually assumed the indefinite outlines of three paramecia (Fig. 16, *i*) but when finally killed on the ninth day these outlines were again lost (*j*). The macronuclei were properly distributed for three individuals but the micronuclei were increased in number, six perfect ones being found, separated by considerable distance from the macronuclei.

In experiment no. 25 not only were normal individuals formed but they were detached and lived for some days as ordinary paramecia. The original vegetative cell was cut in zone II. on January 28, leaving the nucleus, the lower part of the peristome and the mouth intact (Fig. 20, *a*, *b*). On the twenty-ninth the fragment divided in the original center of the cell forming a small truncated anterior fragment and a normal posterior cell (Fig. 20, *c*, *d* and *e*). The normal cell, as in experiment 22 above, divided on the following day forming normal cells, and these continued to divide normally on the average of once a day throughout the life of the other fragment and were discarded on death of the latter. The anterior truncate cell (*e*) grew much larger during the 48 hours after the operation and the 24 hours after division, and attempted to divide on the thirtieth, the same day that its normal sister cell divided. The result of this effort was a monster with two mouths and two vacuoles (Fig. 20, *f*). On the first of February, or the fourth day after the operation, the monster attempted a second division, this time a double division, the result being one free individual and a monster with three mouths (Fig. 20, *h*, *g*). This free individual was smaller than the normal but it lived for 48 hours and died during the process of division. It was detached from the parent mass at the point corresponding to the upper end of Fig. 20, *g*. The monster, meanwhile, continued to grow in size and on the sixth day had two perfect *Paramecium* buds attached by their posterior ends, and several papilla-like outgrowths which continued to grow like buds until on the ninth day well-defined *Paramecium* cells were formed but remained attached to the mass of protoplasm (Fig. 20, *i*, *j*). On the ninth day a second normally formed cell (*k*) was detached, this being the upper one figured in 20, *i*. This free form did not attempt to divide but died before the end of 24 hours. On the twelfth day, still another free form was given off (Fig. 20, *n*), this being the individual on the left side of Fig. 20, *l*. This one was small like all of the detached forms, but appeared to be normal, living for 36 hours. On the tenth and eleventh days the outlines of the previously distinct paramecia buds on the parent mass, became obscure and the individuals seemed to fuse with the protoplasmic mass while the latter twisted and turned with the various ciliary movements. At one period on the tenth day the mass became drawn out into two main lobes (*l*) with a connecting strand between them and appeared to be dividing, but the figure was due to the mechanical pressure caused by the presence of a girdle of zoöglœa about the middle. When this girdle was removed with needles, the protoplasm returned to the more solidly massed state (*m*). A similar constriction appeared on the thirteenth and fourteenth days and zoöglœa was once more removed and the obscure outlines of ghostly paramecia again appeared, no less than eight bizarre forms stretching out from the parent mass at one time while six more mouths could be counted on the general surface (Fig. 20, *o*).

On the following day or fifteenth day after the operation, two dead masses of protoplasm were found; the monster (Fig. 20, *o*) had divided in the middle and the two separated masses evidently had not been physiologically balanced, and had died.

In preparations made with the dead masses no definite nuclei could be distinguished, the material had evidently died during the night and had been dead too many hours for the preservation of the nuclei. A large granular mass in one of the fragments may have been the remains of nuclei perhaps aggregated into a mulberry mass as described by Balbiani.

GENERAL.

Balbiani's conclusion that *Paramecium* does not regenerate as do other protozoa is too sweeping a generalization. A small percentage of cases do regenerate, but the percentage varies with different races. In the experiments here outlined, three different giant races of *Paramecium caudatum* were used. One race, from brackish water in Roscoff, gave approximately 10 per cent. of regenerations; another race, from New York, gave approximately 1 per cent.; another race, also from New York, gave a relatively high percentage of regenerations, approximately 30 per cent., and in a fourth race now under examination every one—or 100 per cent.—regenerates. The method employed by Balbiani was to cut *en masse* instead of individuals one by one. This method made it impossible to determine twenty-four hours afterwards, whether the perfect cells had not been cut at all, or had been cut and had regenerated.

From my results it appears, therefore, that different races have different powers of regeneration, or, stated in another way, have varying degrees of vitality. This power of regeneration is connected in some way, apparently, with the physical make-up of the protoplasm. In other forms of protozoa, notably in *Stentor* (many observers, including Balbiani, Gruber, Nussbaum, Verworn, Prowazek, Lillie, Popoff and others), in *Loxophyllum* (Holmes), in *Stylonychia*, *Oxytricha*, *Spirostomum*, *Frontonia*, *Trachelocerca*, *Dileptus* and *Spathidium*, all of which, save *Loxophyllum*, I have experimented with in this connection, the protoplasmic walls come together more or less quickly after the operation of cutting, thus leaving no endoplasm exposed. In *Stentor*, *Spirostomum*, *Trachelocerca*, *Spathidium*, *Dileptus* and *Frontonia*, the walls come together in a relatively short time, but in *Stylonychia* and *Oxytricha*, the union is delayed and sometimes never occurs. In *Paramecium*, on the other hand, with the exception of the fourth race mentioned above, the cut fragments form a new membrane upon the cut surface and the normal form is not regained, the cell dividing asymmetrically. Where the vitality is sufficiently strong for regeneration the normal form is occasionally gained before division of the fragment, but more often after a gradual change requiring several generations. In the fourth race, on the other hand, the walls of the fragments behave exactly as in *Stentor* or *Loxophyllum* and regeneration is perfect.

The difference in regenerative power may be due to the differences in potential at different periods of the life cycle, and correlated with differences in the physical make-up of the protoplasm at such periods. This certainly appears to be the case with the power to divide, abnormal divisions or monster formation taking place at the end of a long series of generations (see Calkins, 1904), and in those races of *Paramecium* where regeneration occurs in a small percentage of cases.

Child and others have concluded that movements have much to do with the restoration of form in regenerating animals. Holmes shows that this is of secondary importance in the regeneration of cut fragments of *Loxophyllum*. So too in *Paramecium*, movement apparently plays no part whatsoever in the restoration of form. The old peristome is always the site of the new mouth formation, but in the majority of cases, if the mouth is cut away, the anterior fragment with the bulk of the peristome fails entirely to form a new mouth. The general shape of the cell is usually retained; there is no rounding out of the cut fragment any more than there would be were the *Paramecium* made of cheese. Form, therefore, is a highly stable characteristic of *Paramecium*. This is demonstrated with remarkable clearness in the case of monsters. A two mouth monster is, at first, little more than an elongate amorphous mass of protoplasm (Figs. 14, 15, 20). As it grows older, however, the typical shape of the organism begins to appear. Still more remarkable is the budding out of individual forms in monsters of older growth. Here, as the mass is watched from day to day, fairly complete cells make their appearance only to be absorbed again in the general mass. This absorption is analogous to that resulting from a wounded *Paramecium* in which the cut failed to extend entirely through the diameter of the cell. In such cases the wound is closed and the cut surfaces fuse within a short time. Experiment no. 25 (Fig. 20) is a good illustration of the appearance and disappearance of individuals from the common protoplasmic mass of such a monster, the buds always growing into a semblance at least of the typical *Paramecium*.

Form in *Paramecium*, therefore, is a persistent characteristic, apparently independent of movement, and connected in some way with the physical make-up of the protoplasm.

Regeneration of the organism which occurs in a small percentage of cases varying in different races, is probably due to a perfectly balanced physiological condition of the cell. This conclusion is deduced from the fact obtained in many experiments, that a comparatively insignificant cut whereby only an extremity of a *Paramecium* is removed, has a profound effect upon the further activities of the cell. Examples are given in experiments of Table I. and of Table IV., where one or the other end of the cell was removed. In the majority of cases the residual fragment died within a short period; in a few cases the residual fragment divided asymmetrically, giving rise to one normal *Paramecium* which continued to live and to multiply normally, and an abnormal cell which soon died; in a few cases again, the fragment regenerated either before or soon after division.

If this phenomenon could be explained we should be well on the way to an explanation of that ghost-train of processes which we designate vitality. To speak of a balancing of processes, or of a stable and unstable equilibrium, however, is no explanation of what takes place. Some recent observers have undertaken the task of explaining cell physiology, including division, through the varying ratio of nuclear mass to cytoplasmic mass. Balbiani believed that monsters are due to some slight injury to the macronucleus in cutting, and made the generalization that they are "always individuals mutilated in the anterior part whose descendants give rise to abnormalities. . . . Those which lose the posterior part only, always multiply normally by fission. . . . A sort of paralysis attacks cells thus injured in their nuclei" ('92, p. 78).

The number of monsters (four) obtained by Balbiani is too small to support such a generalization. My experiments, in which five of the thirteen monsters were derived from cells cut in the posterior half throw this suggestion out of further consideration.

A more modern view of the nature of physiological processes of the cell was suggested by R. Hertwig ('03) and has been elaborated by himself and his school in numerous publications. This theory according to Popoff's ('08) presentation, involves the view that the quotient obtained by dividing the cytoplasmic mass by the nuclear mass is fairly constant under "normal" conditions, and just as long as this relation varies only within narrow limits, the cell functions are normal. But if by disproportionate growth of either cytoplasm or nucleus, the nucleus-protoplasm relation is changed to favor either one, then the cell gets into an "abnormal" condition. In order to become "normal" again, the "normal" nucleus-protoplasm relation must be reëstablished. Cell division, Hertwig believes, is the means whereby normal relations are reëstablished. He postulates, furthermore, two periods in the growth of the nucleus; one "functional," the other "divisional," the former beginning shortly after division of the cell and lasting until shortly before the following division, the nucleus growing less rapidly than the cytoplasm and disturbing the nucleus-protoplasm relation in favor of the cytoplasm. This disturbance of "normal" relations persists up to a certain point which Hertwig calls the nucleus-protoplasm-tension-moment (Kernplasmaspannungsmoment), which he regards as the immediate inciting cause of cell division, the first effect being the rapid "division growth" of the nucleus. The result of the division thus started is a return to the "normal" nucleus-protoplasm relation.

The use of terms normal and abnormal in this connection is hardly appropriate, for cell division is certainly a normal process and all stages leading to it must likewise be normal, hence an abnormal nucleus-protoplasm relation in a normal vegetative cell, cannot exist. Waiving this verbal matter, however, and examining the principle involved, we admit the change in the relative masses of nucleus and cytoplasm in periods of growth. As to the growth of the nucleus prior to cell division the evidence is not so clear. In *Uronychia transfuga* at division, for example, not only is the exposed surface of the nuclear elements lessened but the mass as well is decreased, and the two small cells resulting from the division contain small nuclei which subsequently break up into fragments before growth commences and with growth there is a constantly increasing surface of nuclear matter (Calkins, '11). On the Hertwig theory we should expect a restitution by division, of the "normal" mass relations in fragments of cells containing either a preponderance of nuclear matter or of cytoplasmic. Fragments of cells, however, show no such regulatory process, for cutting the cells invariably postpones division unless the division is already under way. Under abnormal conditions of temperature Popoff finds that the volume of the nucleus increases more rapidly than that of the cytoplasm. Various observers have noted that under abnormal conditions, generally, including that of weakened vitality from any cause, the nucleus is relatively larger than under normal conditions and the enlargement is better interpreted as an effect of lessened vitality than its cause. Popoff likewise performed cutting experiments on *Frontonia leucas* and found, as Balbiani and others have found, that the cell, if cut late in an inter-divisional period, divides in the original plane of division. But if the cell is cut before this period the cell first regenerates before dividing. He concludes: "Diese Versuch zeigen, dass der Anstossgebende Moment der Theilung in dem Augenblick der Kernplasmaspannung zu suchen ist. So wie die Zelle diesen Moment uberschritten hat, ist der Teilung derselben schon eingeleitet und kein ausserer Eingriff kann sie mehr verhindern" ('08, p. 337). It is difficult to see this conclusion from the facts presented and still more difficult to interpret such cases as my experiment 10, Table III., where two successive divisions of a fragment were entirely asymmetrical (see p. 52). In this case furthermore there was a long period between divisions when "normal" conditions of the division energy might have been reëstablished.

In reference to my *Paramecium* work of 1904 Popoff states that at the final period of depression the nucleus-protoplasm relation was changed to the advantage of the nucleus and he argues that this condition may have been the cause of the depression (*loc. cit.*, p. 339). As my paper of 1904 makes no reference to the nucleus-protoplasmic relation he drew his conclusion from the photographs of the cells at the end period of depression, but naturally, he did not realize that when the cells thus photographed were killed, they had lived for days without dividing. Under such conditions, like any other *Paramecium* cell under similar conditions, they were "abnormal" so far as the nucleus-protoplasm relation is concerned.

In the latter part of the first section of Popoff's paper of 1908 he gives an inkling of a possible interpretation of the nucleus-protoplasm relation in the statement: "Die Kernplasmarelation wird ein morphologischer fassbarer Ausbruck der jeweiligen Chemismus der Zelle bleiben." With this interpretation we are inclined to agree, and we look upon the changeable nucleus-protoplasm relation as an unstable effect produced by varying conditions of nutrition, temperature, or vitality and not at all as a cause of division or depression or of vitality. In a mutilated *Paramecium* the nucleus divides equally, the cell unequally; the smaller fragment has a full size nucleus and a much deranged "nucleus-protoplasm" relation; yet, in some cases at least, it behaves like a normal cell, dividing at the proper time and again forming dissimilar products, one of which is perfectly normal, the other again abnormal (expt. 25, p. 57).

By removing a portion of a cell there can be little doubt that the ordinary chemical interchange, or perhaps the physical or electrical potential, of cell and nucleus is violently disturbed. In forms with a labile protoplasm, as manifested in some forms of *Paramecium* and as in *Stentor*, *Loxophyllum Spathidium*, etc., the wounded cell regenerates quickly, but in other races of *Paramecium* and in non-nucleated fragments of other protozoa, the more stable protoplasm does not respond and regeneration fails. Division of the fragment indicates that the power of division and the power of regeneration are entirely independent phenomena and are alike independent of the nucleus-protoplasm relation so far as mass is concerned. The failure of a small fragment to divide completely might be due to the fact that the division energy is not great enough to overcome the surface tension of the body walls. But this interpretation would hardly account for the incomplete division of the cell in which only a small portion of the terminal protoplasm is removed (experiment 7, Table IV., p. 52).

Summary.

1. *Paramecium caudatum* may be easily cut with a scalpel, one, the larger, part, continues to live in about 30 per cent. of cases, and both parts never for more than 24 hours. If the nucleus is injured by the knife neither part lives for more than a few hours. The present paper is a summary of about 150 recorded experiments.

2. Cells cut at either extremity are just as much demoralized as those cut in apparently more vital parts.

3. The power of regeneration varies in different races of "giant" *Paramecium*. In one race only about 1 per cent. regenerated; in another race about 10 per cent., in a third race about 30 per cent. regenerated, and in a fourth race all, or 100 per cent. regenerated. This last race is not included in the present paper.

4. There is strong evidence of a division zone in *Paramecium* which lies in the center of the cell. If the cell is cut anterior or posterior to this zone the fragment divides in the original plane into a truncated abnormal form and a normal form. The truncated form may divide again not through its center, but through the center of the cell were it perfect.

5. A fragment, whether anterior or posterior, dies without division in the majority of cases; in cases of fission, it divides asymmetrically as stated above. The division, however, is retarded. In a few cases the division is abortive and a monster results.

6. After such a division into an abnormal and a normal cell, the normal cell continues to divide normally and forms a race of

individuals entirely unaffected by the operation. The abnormal cell in some cases, divides again asymmetrically and forms another normal cell and an abnormal cell; in other cases the second division is abortive and monsters are formed; in a few cases it continues to divide with a gradually decreasing abnormality until normal forms are regained; in many cases, finally, it dies without further division.

7. The monsters represent as many individuals as there are mouths. In one monster as many as fourteen mouths were present at one time. There is a well-marked tendency of the protoplasm to assume the form of a normal *Paramecium* about each of the mouths, and such individuals bud out of the protoplasmic mass, remain for several hours, and may be absorbed again into it.

8. Free cells, complete in all respects, may be given off from the protoplasm of a monster. These may live for days and may even divide, but vitality is weak and they invariably die.

9. Cell division and cell regeneration are entirely independent phenomena. A fragment divides without regenerating and the abnormal product of this division may divide again without regenerating. In other cases the fragment divides asymmetrically and the resulting abnormal cell regenerates before the following division. The cell need not regenerate to divide, and may or may not regenerate before dividing.

10. The *Paramecium* cell acts as a unit; cytoplasm and nucleus are equally important, a small loss at either extremity is usually enough to throw the physiological mechanism out of gear and lead to death, to asymmetrical division, or to monster formation.

11. The phenomena resulting from cutting *Paramecium* cannot be explained on the Kernplasmarelation theory of the Munich school. Mass of nucleus in relation to mass of protoplasm is only a morphological index of the physiological (chemical, electrical) activity of the cell—an effect and not a cause of the various vital reactions.

12. Regeneration of a cut cell in races with a limited power of regeneration, is more often observed in cells that have recently conjugated, or in cells that were cut while in conjugation, than in ordinary vegetative cells.

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DESCRIPTION OF PLATES.

Unless otherwise stated, all figures are made from camera drawings or sketches from the living cells. The black lines indicate the planes of cutting. All figures are of *Paramecium caudatum.*

PLATE I.

FIG. 1. *a*, vegetative cell cut in zone 1; *b*, the fragment at the end of four hours. It died within 24 hours. Experiment 11, Table I.

FIG. 2. *a*, a similar cell cut as above; *b*, fragment 72 hours afterwards showing neither regeneration nor growth. Experiment 4, Table I.

FIG. 3. *a*, conjugating *Paramecium* cut in zone 2; *b*, fragment 24 hours after the operation; *c*, the other, normal, exconjugant; *d*, the fragment fixed and stained after 96 hours, showing normal vegetative nuclei indicating that conjugation had just begun when cut. Experiment 2, Table II.

FIG. 4. *a*, vegetative cell cut in zone 2; *b*, the fragment 6 hours after cutting; *c*, the fragment dividing 24 hours after cutting and killed during division to show the nuclear apparatus. Experiment 7, Table II.

FIG. 5. *a*, vegetative cell cut in zone 2; *b*, fragment dividing, 16 hours later; *c*, anterior, and *d*, posterior, cells killed 24 hours after division for the nuclear structures. The disintegrated fragments of macronuclei indicate recent conjugation. Experiment 9, Table II.

FIG. 6. *a*, vegetative cell cut in zone 2; *b*, fragment 24 hours after cutting, with tentacle-like process; *c*, fragment dividing in the original center of the cell 48 hours after cutting; *d*, *e*, resulting daughter cells 6 hours after division. Both of these cells died on the following day. Experiment 21, Table II.

FIG. 7. *a*, vegetative cell cut in zone 2; *b*, fragment 72 hours after cutting; *c*, fragment in division on the fourth day; *d*, *e*, daughter cells resulting from this division; *f*, cell *d* on the sixth day, much grown; *g*, *h*, daughter cells of *f* on the ninth day; *i*, *j*, daughter-cells of *h* on the twelfth day; *k*, *l*, daughter cells of *g* on the twelfth day; *m*, *n*, figures of *i* and *k* on the seventeeth day when they died; *o*, figure of *j* on the eighteenth day after the operation when it, too, died. Experiment 54, Table II.

FIG. 8. *a*, vegetative cell cut in zone 2 near the plane of division; *b*, division of the fragment 24 hours after cutting; *c*, *d*, resulting cells, the former minute, the latter normal. Both died on the second day. Experiment 29, Table II.

FIG. 9. *a*, vegetative cell cut in zone 3; *b*, fragment 12 hours after cutting; *c*, same dividing 24 hours after cutting; *d*, *e*, daughter cells resulting from division of *b*; *f*, division of truncated cell *e* on the fourth day; *g*, figure of truncated posterior half of *f* on the eighth day; *h*, *i*, daughter cells of the normal half of *f*, on the tenth day, *j*, figure of *g* on the tenth day. Experiment 19, Table III.

FIG. 10. *a*, dividing cell cut in zone 3; *b*, division figure after removal of the anterior end; *c*, small anterior cell after division; *d*, normal posterior cell after division of *b*; *e*, figure of *c* 24 hours later; *f*, figure of *d*, 24 hours after division of *b*. Experiment 11, Table II.

FIG. 11. *a*, dividing cell cut in zone 4; *b*, posterior fragment with mass of crystals in the anterior end. This died on the following day. Experiment 5, Table IV.

FIG. 12. *a*, ex-conjugant cut in zone 3; *b* and *c*, the anterior fragment 48 and 96 hours after cutting.

PLATE I.

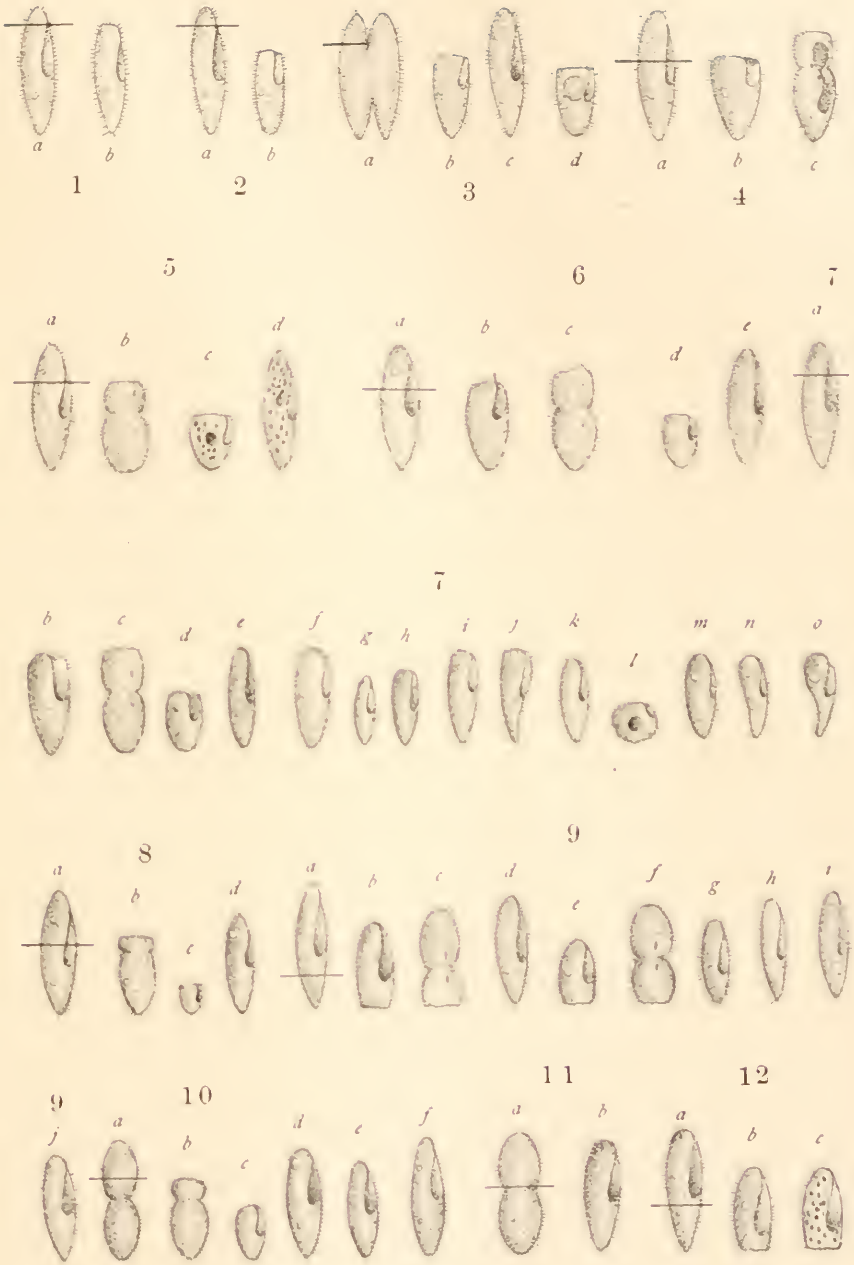

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Plate II.

Fig. 13. *a*, vegetative cell cut in zone 3; *b*, fragment three days after the operation; *c*, asymmetrical division on the fourth day; *d*, same on the seventh day; *e*, same on the eighth day. Dead on the ninth day. Experiment 23, Table III.

Fig. 14. *a*, vegetative cell cut in zone 2; *b*, fragment 3 days after the operation; *c*, monster from asymmetrical division on the fourth day; *d*, same on the fourteenth day. Dead on the seventeenth day. Experiment 5, Table II.

Fig. 15. *a*, vegetative cell cut in zone 3; *b*, asymmetrical division of the fragment on the following day; *c*, *d*, normal and abnormal cells resulting from the division; *e*, monster formed by the cell *d* on the third day after the operation. Dead on the fifth day with nuclear apparatus normal. Experiment 17, Table II.

Fig. 16. *a*, vegetative cell cut obliquely in zone 2; *b*, asymmetrical division of the fragment 24 hours later; *c*, *d*, abnormal and normal cells resulting from this division; *e*, abnormal cell *c* on the fourth day; *f*, *g*, normal cells resulting from normal fission of *d* on the third day; *h*, monster formed from *e* on fifth day; *i*, second attempted division of the monster on the the sixth day; *j*, same monster after fixation and staining on the ninth day. Experiment 22, Table II.

Fig. 17. *a*, vegetative cell cut in zone 2; *b*, attempted division of the fragment 48 hours after the operation; *c*, same monster on the seventh day, 24 hours after addition of nuclein; *d*, same monster on the ninth day after a second (double) attempted division; *e*, free, truncate fragment derived from the upper extremity of the monster figured in *d*; *f*, *g*, two monsters derived from the division of monster *d* at the middle point (*x*) on the tenth day; in *f* the lower branch is in the process of division; *h*, small, reversed, truncate fragment 24 hours after being formed from division of the lower branch of *f*; *i*, *j*, the two monsters on the eleventh day; *k*, the smaller monster after fixation and staining on the fourteenth day; *l*, the larger sister monster after fixation and staining on the twentieth day after the original operation. Experiment 40, Table II.

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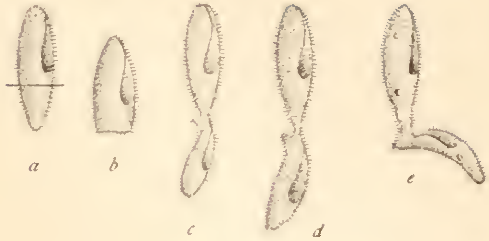

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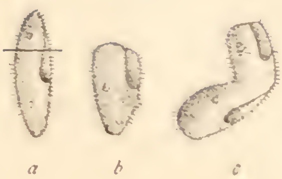

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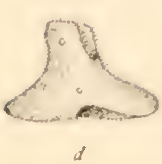

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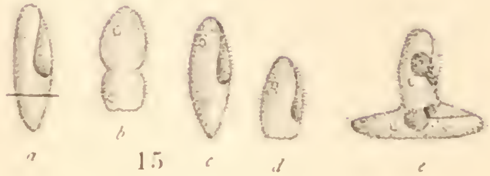

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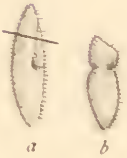

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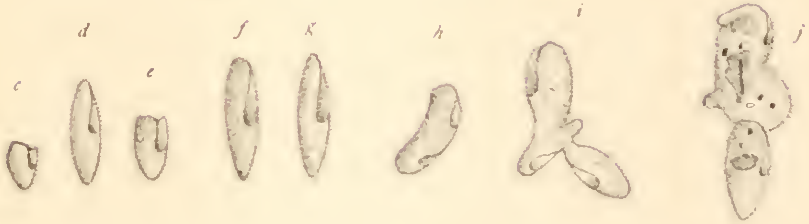

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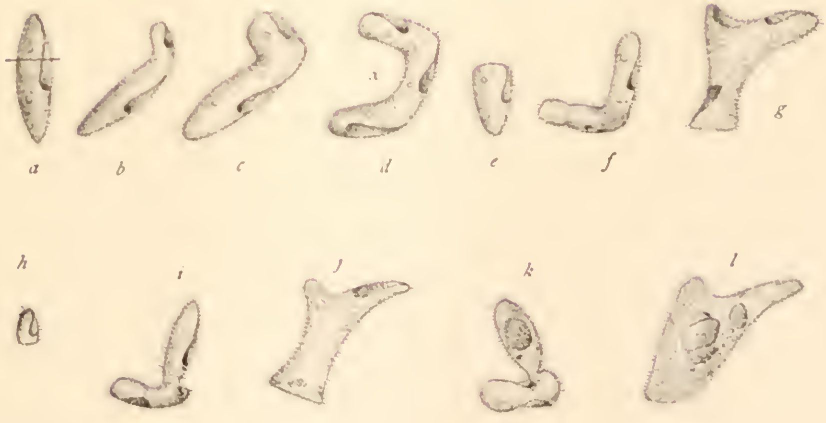
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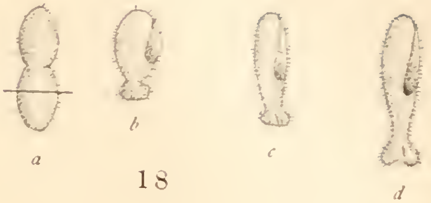
PLATE III.

FIG. 18. *a*, dividing cell cut in zone 2 of the posterior cell; *b*, fragment 24 hours after the operation; *c*, fragment 3 days after the operation. Experiment 6, Table II.

FIG. 19. *a*, vegetative cell cut in zone 3; *b*, anterior fragment 48 hours after the operation; *c*, attempted division resulting in monster formation on the third day after cutting. Experiment 6, Table III.

FIG. 20. *a*, vegetative cell cut in zone 2; *b*, posterior fragment 12 hours after the operation; *c*, asymmetrical division of *b*, 24 hours after the operation; *d*, *e*, normal and abnormal cells resulting from the division of *c*; *f*, attempted division of *e* 48 hours after the operation; *g*, *h*, results of a second (double) division of *f* on the fourth day; *h*, a normal *Paramecium* derived from the upper branch of *g*; this lived for 48 hours and attempted to divide but died in the process; *i*, monster derived from *g* on the sixth day, with two practically complete paramecia and several papilliform buds; *j*, same monster on the ninth day; *k*, a detached free cell on the ninth day derived from the upper cell shown in figure *i*; *l*, same monster on the twelfth day with constriction due to a girdle of zoöglœa; *m*, same monster on the thirteenth day with individuals partially withdrawn; *n*, a third, free individual given off on the twelfth day, this being the attached individual on the left side of Fig. *l*; *o*, same monster on the fourteenth day with eight *Paramecium* buds, six mouths additional (not shown) and ten vacuoles. As the figure shows the mass was again constricted and this time the constriction resulted in division but the daughter monsters were dead on the next day. Experiment 25, Table II.



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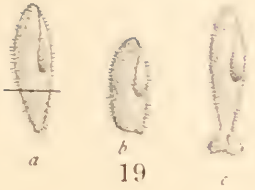

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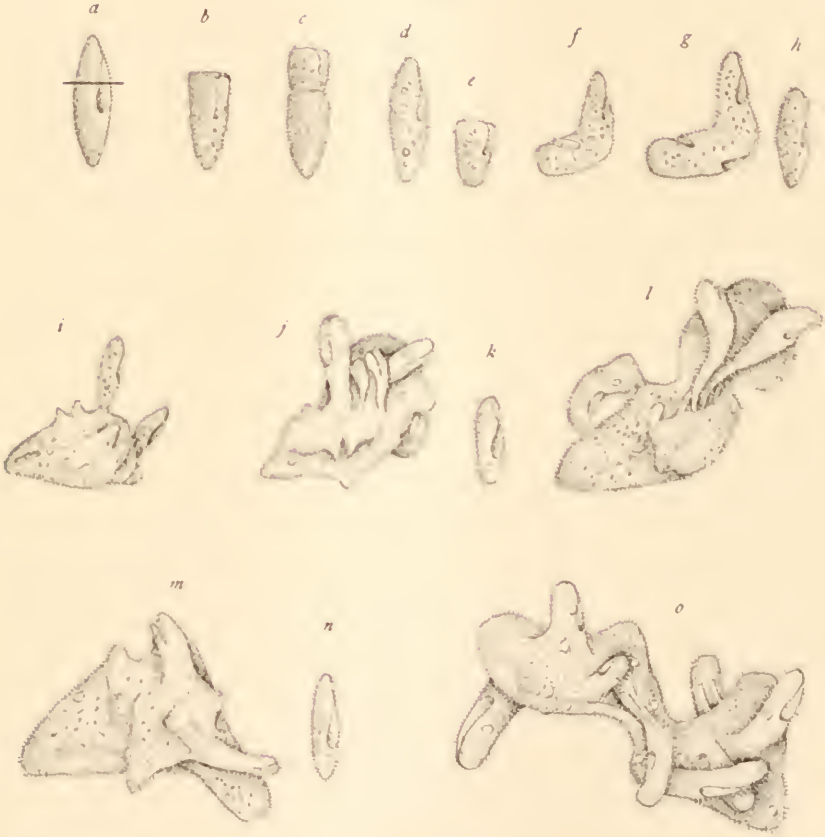
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BIOLOGICAL BULLETIN

I. TRUSTEES

AUGUST, 1910

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GEORGE LEFEVRE	University of Missouri.
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A. P. MATHEWS	University of Chicago.
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GEORGE T. MOORE	Washington University, St. Louis.
L. L. NUNN	Telluride, Colo.
JOHN C. PHILLIPS	299 Berkeley Street, Boston, Mass.
C. O. WHITMAN[1]	University of Chicago.

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[1] Deceased.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord ONE THOUSAND, EIGHT HUNDRED AND EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio,* members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio.*

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to a dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT

For the Year Ending December 31, 1910

Income

Annual dues		$ 606.00	
Donations (Charles R. Crane)		8,500.00	
Miscellaneous:			
Interest on deposit	$ 38.89		
Rent of microscopes	14.00		
Bath house	52.40		
Div. on 35 shs. Woods Hole Yacht Club	350.00		
Mosquito survey	400.00		
Check not used	.82	856.11	
Supply department		9,300.58	
Mess (net)		551.97	
Tuitions		4,150.00	
			$23,964.66

Expenses

Administration	$ 900.00
Advertising	99.31
Biological Bulletin (net)	512.70
Boats and launch	1,502.77
Botany	10.05
Chemicals, chemist supplies, etc.	783.23
Fish trap	88.35
Instructors' salaries	3,180.00
Insurance	284.35
Interest	150.00
Labor	3,872.52
Mosquito survey	50.00

Periodicals and library supplies	789.60	
Postage	89.06	
Repairs	1,377.09	
Sundries	1,900.20	
Supply department	6,958.25	$22,547.48
Surplus		$ 1,417.18

ITEMIZED LIST OF SUNDRY EXPENSES FOR THE YEAR 1910

Announcements and expense mailing	$110.80
Ash cans	12.00
Care of cemetery lot	2.00
Carting ashes	33.95
Covering for ice	8.66
Dr. Drew's office expenses	225.78
Dr. Lillie's office expenses	104.00
Exchange on checks	31.70
Express	47.27
Freight and hauling	6.80
Gas machine expense	55.12
Howes' bill	36.64
Hubbard, J. G., services	60.00
Instruments, laboratory supplies	178.40
Laundry	21.36
Lecture expense	22.30
New York Calcium Light Company	7.38
Notary fees	3.00
Petty cash at Woods Hole	63.02
Premium on bond	7.50
Rent of microscopes	30.00
Rent of typewriter	9.00
Spring water	10.50
Stationery, office supplies	57.50
Steel hand cart	10.00
Sundry supplies	37.57
Teaming	2.20
Telephone	31.89
Travelling expenses	39.50

Treasurer's office, clerical services	520.00	
Trustees' dinner	16.00	
Veeder's expenses	24.76	
Water	65.00	
Wooden cases	8.00	
		$1,900.20

MARINE BIOLOGICAL LABORATORY INVESTMENTS

JANUARY 1, 1911

Reserve Fund

Amount of fund December 1, 1899	$4,553.14	
Received from life memberships	600.00	
Income to January 1, 1911	2,090.15	
Gain from sale of securities	372.20	
	7,615.49	
Paid for current expenses of Laboratory	6,000.00	$1,615.49
Reserve fund now consists of the following:		
$3000 Am. Tel. & Tel. Co. 4s cost	$2,921.25	
6 shs. Am. Smelting & Refining Co. Pfd. cost	732.00	
5 shs. General Electric Co. cost	756.25	
Cash	205.99	
	4,615.49	
The above stocks and bonds are held as collateral for loan of	3,000.00	$1,615.49

Library Fund

Amount of fund December 1, 1899	$ 866.15	
Income to January 1, 1911	701.47	
Gain from sale of securities	74.88	
Sale of rights	.81	$1,643.31
Library fund now consists of the following:		
3 shs. Am. Tel. & Tel. Co. cost	$ 383.25	
4/5 of $1000 Am. Tel. & Tel. Co. 4s cost	779.00	
1 sh. Am. Smelting & Refining Co. Pfd. cost	122.00	
2 shs. General Electric Co. cost	302.50	
Cash	56.56	$1,643.31

Lucretia Crocker Fund

Amount of fund December 1, 1899	$2,500.00	
Income after paying students' fees	522.41	
Sale of rights	1.90	$3,024.31

Lucretia Crocker Fund now consists of the following:

18 shs. Vermont & Mass. R. R. Co. cost	$2,416.50	
1 sh. West End St. Ry. Co. cost	83.00	
1 sh. Am. Tel. & Tel. Co. cost	127.75	
1/5 of $1000 Am. Tel. & Tel. Co. 4s cost	194.75	
1 sh. General Electric Co. cost	151.25	
Cash	51.06	$3,024.31

V. THE DIRECTOR'S REPORT

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I have the honor to submit herewith a report of the twenty-third session of the Marine Biological Laboratory for the year 1910, and some recommendations for future development.

We record with sorrow the loss by death during the year of three members of the board of trustees: Dr. C. A. Herter, Professor D. P. Penhallow and Professor C. O. Whitman. It is fitting that due expression of the sense of our loss should be given in the form of resolutions to be spread upon the minutes of the board and sent to the families of our lamented colleagues. The death of Professor Whitman was sudden and unexpected. He was taken with pneumonia on November 30 and died on December 6. Memorial services in his honor were held at the University of Chicago on December 8, and his body was laid in the laboratory lot in the Episcopal Cemetery at Woods Hole on December 10 in the presence of a small gathering of his former associates and friends, who alone were able to attend.

Professor Whitman's death may be said to mark the close of the first period of development of the Marine Biological Laboratory. As director for the first twenty-one years of the existence of the Laboratory, more than any other person he gave the Laboratory its status and its ideals. In a very fitting way, therefore, we may say that the Laboratory is a monument to his memory. But it is desirable and due that some permanent memorial should mark his last resting place near the institution that he loved so well, and it is recommended that a committee be appointed to take this matter in charge.

The staff of instruction in 1910 comprised 29 members, representing eighteen institutions. Acknowledgment should again be made to the members of the staff of investigation who rendered their services freely.

The attendance of workers at the laboratory comprised 62 investigators and 64 students—a total of 126 as compared with 66 investigators and 63 students, total, 129, in 1909, and 52 investigators, 48 students, total 100, in 1908. There was a gratifying increase in the number of subscribing institutions, from 19 in 1909 to 24 in 1910. One of the subscribing institutions of 1909 dropped out this year. The new subscribing institutions were Dartmouth College, Massachusetts Agricultural College, The Rockefeller Institute for Medical Research, The University of Illinois and the Washington University Alumni Association. The membership of the corporation was also somewhat increased. The receipts of the supply department increased from $8.549.55 in 1909, to $9,300.58, in 1910.

No definite progress was made in the plan for a permanent building during the year, except that steps have been taken to secure the possession of more land that is necessary for the protection of our present holdings. Success has been attained in this direction and I think we may say that we are distinctly nearer the realization of the permanent building, not only in point of time but in preparedness. This raises definitely two questions: first, as concerns the nature and use of the building, and second, concerning administration affairs of the laboratory.

As regards the building, I believe that the committee that was appointed December 30, 1909, was fully agreed that the new building should provide for the library and for a certain number of research rooms. This committee has not formally met and I do not present this as an official report from the committee. But on the assumption that provision would be made for research rooms on a more adequate scale and with better equipment than the present quarters, I think that we should begin to ascertain the attitude of certain universities and research institutions with reference to the maintenance of quarters for investigation to be available throughout the year. Such an inquiry may seem premature; but when we consider that the character of the building to be erected must depend to a certain extent upon the financial support of institutions for its maintenance, I think it will be admitted that we can not move too soon in the direction of receiving assurances.

The second question concerns administration. As matters now stand the burden of administering the affairs of the laboratory falls rather heavily on men who already have a full assignment of work in their own universities. There is no question but that the work of the laboratory could be made more effective in all respects if we had an administrative officer who devoted his entire time to the affairs of the laboratory; it is also questionable whether the laboratory can continuously retain the services of men under present conditions, when their work is given at the expense of effectiveness in their university work and their research. It is therefore recommended, that, as soon as may be, the office of assistant director be made a resident position for the entire year. Under such an arrangement all our relations should prosper better: with the investigators, with the students, and with institutions. The expense involved would probably be a diminishing amount owing to improvement in laboratory conditions generally.

As soon as plans are definitely under way for a permanent building, such an officer would be necessary to supervise the details decided on by the committee on the building, and to make investigations on behalf of the committee; and later when the laboratory was erected to receive investigators at other seasons of the year besides the summer. He should be furnished with assistance adequate to leave him sufficient time for research.

There are appended as parts of this report (1) a list of the staff, (2) lists of investigators and students in attendance, (3) a comparative tabular view of attendance, (4) the names of subscribing institutions, (5) the evening lectures, (6) a list of members of the corporation.

1. THE STAFF, 1910

F. R. LILLIE, Director,

Professor of Embryology, University of Chicago.

GILMAN A. DREW, Assistant Director,

Professor of Biology, University of Maine.

ZOÖLOGY

I. INVESTIGATION

Zoölogy and Embryology

E. G. Conklin	Professor of Zöology, Princeton University (Absent in 1910.)
Gilman A. Drew	Professor of Biology, University of Maine.
George Lefevre	Professor of Zoölogy, University of Missouri. (Absent in 1910.)
Frank R. Lillie	Professor of Embryology, University of Chicago.
Thos. H. Montgomery, Jr.	Professor of Zoölogy, University of Pennsylvania.
T. H. Morgan	Professor of Experimental Zoölogy, Columbia University.
E. B. Wilson	Professor of Zoölogy, Columbia University.

II. INSTRUCTION

Winterton C. Curtis	Professor of Zoölogy, University of Missouri.
Paul M. Rea	Professor of Biology, College of Charleston, and Director of the Charleston Museum.
Edward E. Wildman	Central High School, Philadelphia.
John W. Scott	Westport High School, Kansas City.
G. S. Dodds	Fellow in Zoölogy, University of Pennsylvania.
H. J. Spencer	Assistant in Zoölogy, Columbia University.

EMBRYOLOGY

I. INVESTIGATION. (See Zoölogy)

II. INSTRUCTION

Gilman A. Drew	Professor of Biology, University of Maine.
Lorande L. Woodruff	Assistant Professor of Biology, Yale University.
William E. Kellicott	Professor of Biology, Goucher College.
Robert A. Budington	Associate Professor of Zoölogy, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

Albert P. Mathews	Professor of Physiological Chemistry, University of Chicago.
E. P. Lyon	Professor of Physiology, University of St. Louis.
R. S. Lillie	Instructor in Comparative Physiology, University of Pennsylvania.

II. INSTRUCTION

H. H. Newman	Professor of Zoölogy, University of Texas.
Frank P. Knowlton	Professor of Physiology, Syracuse University.
F. H. Pike	Instructor in Physiology, University of Chicago.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

Edward G. Spaulding	Assistant Professor of Philosophy, Princeton University.

BOTANY

George T. Moore	Professor of Plant Physiology and Applied Botany, Washington University.
George R. Lyman	Assistant Professor of Botany, Dartmouth College.

B. M. Duggar	Professor of Plant Physiology, Cornell University.
I. F. Lewis	Professor of Biology, Randolph-Macon College.
Lewis Knudson	Instructor in Plant Physiology, Cornell University.

LIBRARY

H. McE. Knower	University of Cincinnati, Librarian.

CHEMICAL SUPPLIES

Oliver S. Strong	College of Physicians and Surgeons, Chemist.

G. M. Gray	Curator of Supply Department.
Thomas M. Douthart	Collector in Zoölogy.
Arthur W. Taylor	Collector in Botany, Salem High School, Salem, Mass.
John Veeder	Cockswain.

2. INVESTIGATORS

1910

OCCUPYING ROOMS

1. ZOOLOGY

Addison, W. H. F., University of Pennsylvania.

Beckwith, Cora J., Instructor in Biology, Vassar College.

Browne, Ethel N., Instructor, Bennett School, Millbrook, New York.

Budington, Robert A., Associate Professor of Zoology, Oberlin College.

Cary, L. R., Princeton University, Princeton, N. J.

Clark, Eliot R., Associate in Anatomy, Johns Hopkins University.

Curtis, W. C., Professor of Zoology, University of Missouri.

Dodds, Gideon S., Professor of Biology, St. Louis University Medical School, St. Louis, Mo.

Drew, Gilman A., Professor of Biology, University of Maine.

Harrison, Ross G., Bronson Professor of Comparative Anatomy, Yale University.

Harvey, E. Newton, Columbia University, New York City.

Jarvis, May M., Tutor in Zoology, University of Texas.

Kellicott, William E., Professor of Biology, Goucher College.

Knower, Henry McE., Professor of Anatomy, University of Cincinnati.

Lillie, Frank R., Professor of Embryology, University of Chicago.

Loeb, Leo, Assistant Professor of Experimental Pathology, University of Pennsylvania.

McIndoo, Norman E., Fellow in Biology, University of Pennsylvania.

Montgomery, T. H. Jr., Professor of Zoology, University of Pennsylvania.

Morgan, T. H., Professor of Experimental Zoology, Columbia University.

Murbach, Louis, Central High School, Detroit, Michigan.

Patterson, J. Thomas, Instructor in Zoology, University of Texas.

Quackenbush, L. S., Columbia University.

Rea, Paul M., Professor of Biology, College of Charleston.

Scott, John W., Westport High School, Kansas City, Mo.

Spencer, Henry J., Assistant in Zoology, Columbia University.

Spaulding, Edward G., Assistant Professor of Philosophy, Princeton University.

Strong, O. S., Instructor in Anatomy, College of Physicians and Surgeons, New York City.

Watkinson, Grace B., Columbia University.

Werber, Ernest I., Assistant in Anatomy, Johns Hopkins University.

Wells, H. G., Associate Professor of Pathology, University of Chicago.

Wildman, Edward E., Central High School, Philadelphia, Pa.

Wilson, E. B., Professor of Zoölogy, Columbia University.
Woodruff, L. L., Assistant Professor of Biology, Yale University.

2. PHYSIOLOGY

Donaldson, H. H., Professor of Neurology, Wistar Institute.
Hirschfelder, Arthur D., Associate in Medicine, Johns Hopkins Medical School.
Knowlton, Frank P., Professor of Physiology, Syracuse University, College of Medicine.
King, W. O. Redman, Rockefeller Institute for Medical Research, New York City.
Loeb, Jacques, Rockefeller Institute for Medical Research, New York City.
Lillie, R. S., Instructor in Physiological Zoölogy, University of Pennsylvania.
Mathews, A. P., Professor of Physiological Chemistry, University of Chicago.
Newman, H. H., Professor of Zoölogy, University of Texas.
Pike, Frank H., Instructor in Physiology, University of Chicago.
Rogers, Charles G., Associate Professor of Physiology, Syracuse University.
Wasteneys, Hardolph, Rockefeller Institute for Medical Research, New York City.

3. BOTANY

Bessey, Ernest A., Professor of Botany, Michigan Agricultural College.
Duggar, Benjamin M., Professor of Plant Physiology, Cornell University.
Knudson, Lewis, Instructor in Plant Physiology, Cornell University.
Lewis, Ivey Foreman, Professor of Biology, Randolph-Macon College.
Lyman, George R., Assistant Professor of Botany, Dartmouth College.
Moore, George T., Professor of Plant Physiology and Applied Botany, Washington University, St. Louis, Mo.
Thomas, Mason B., Professor of Botany, Wabash College.
Osterhout, W. J. V., Assistant Professor of Botany, Harvard University.
Smith, Erwin F., Pathologist in charge Laboratory of Plant Pathology, U. S. Department of Agriculture, Washington, D. C.

OCCUPYING TABLES

1. ZOÖLOGY

Allyn, Harriet, University of Chicago.
Davis, Sarah Ellen, Student, Columbia University.
Linton, Eleanor A., Washington, Pa.
Kelley, Frank, J., University of Pennsylvania.
Wilson, Herrick E., Assistant in Geology, Oberlin College.
Spooner, Georgiana B., Palo Alto, Cal.

2. PHYSIOLOGY

Donaldson, John Calvert, 3310 Race Street, Philadelphia, Pa.
Tashiro, Shiro, Fellow, University of Chicago.

3. BOTANY

Taylor, Arthur W., Warren, Mass.

STUDENTS

1910

INVERTEBRATE ZOÖLOGY

Baker, Rita G., 72 Huntington Avenue, Boston, Mass.
Bell, Mary M., Oberlin College, Oberlin, Ohio.
Bodwell, Helen E., 137 Main Street, Andover, Mass.
Cashman, Marie E., Syracuse University, Syracuse, New York.
Coar, Herbert G., Dartmouth College, Hanover, N. H.
Eddowes, Florence E., Goucher College, Baltimore, Md.
Estabrook, A. H., Johns Hopkins University.
Fenner, Hattie, University of Kansas, Lawrence, Kansas.
Field, Leanor A., Mount Holyoke College, South Hadley, Mass.
Gavin, Helen, 28 West 97th Street, New York City.
Krantz, Hazel I., Syracuse University, Syracuse, N. Y.
Lee, Horace N., University of Maine, Orono, Maine.
McLaine, Leonard S., Massachusetts Agricultural College.
Martin, Bertha E., Mount Holyoke College, South Hadley, Mass.
Martin, Susie E., Mount Holyoke College, South Hadley, Mass.
Mase, Yae Kemitsaki, Aichiken, Japan.
Mook, Charles C., Columbia University, New York City.
Moon, Evangeline A., 436 Manhattan Avenue, New York City.
Morriss, May J., 485 Central Park West, New York City.
Osgood, Marian S., Mount Holyoke College, South Hadley, Mass.
Paige, Beryl H., Mount Holyoke College.
Patten, Hazel, Goucher College, Baltimore, Md.
Pearson, Leonard S., Academy of Natural Sciences, Philadelphia.
Sevin, Gertrude K., Syracuse University.
Sink, Emory W., University of Michigan, Ann Arbor, Mich.
Topp, Emily, 428 West 20th Street, New York City.
Wilkinson, George N., Bucknell University, Williamsport, Pa.
Williams, Annie E., Cochituate, Mass.
Wiswell, Amy P., East Machias, Maine.
Wright, Coila B., Sheridan, New York.
Zabriskie, Helen M., Vassar College, Poughkeepsie, N. Y.

EMBRYOLOGY

Abbott, Margaret B., 413 West Front Street, Plainfield, N. J.
Appleton, Joseph L., State College, Pa.
Ennis, Agnes A., Barnard College, Columbia University.
Just, Ernest E., Howard University, Washington, D. C.
Kuntz, Albert, State University of Iowa.

Leavitt, George C., University of Maine, Orono, Maine.
Mellen, Ida M., 530 Carleton Avenue, Brooklyn, New York.
Prentiss, Henrietta, Normal College, New York City.
Redelings, Leslie R., Northwestern University, Evanston, Ill.
Smith, Isabel S., Illinois College, Jacksonville, Ill.
Ullman, Henry J., Rush Medical College, Chicago, Ill.

PHYSIOLOGY

Davis, Nathan Smith III. 8 E. Huron Street, Chicago, Illinois.
Marks, Henry S., University of Rochester, Rochester, N. Y.
Stark, Mary B., St. Olaf College.
Stephenson, J. C., University of Chicago, Chicago, Ill.
Whitenton, Robert O., University of Chicago, Chicago, Ill.

BOTANY

1. Plant Structures and Responses

Chandler, Jean Forrest, 4651 North Lincoln Street, Chicago, Ill.
Clement, Frank H. P., Tilton, N. H.
Wells, Collin, Dartmouth College, Hanover, N. H.
Jennison, Harry M., Massachusetts Agricultural College.

2. Morphology and Taxonomy of the Algæ

Brugger, Helen F., Mount Holyoke College, South Hadley, Mass.
Colley, Reginald H., Dartmouth College, Hanover, N. H.
Curtis, Otis F., Oberlin College, Oberlin, Ohio.
Dawson, Ava B., 97 Mountfort Street, Boston, Mass.
Hill, Albert F., Dartmouth College, Hanover, N. H.
Hobbs, Ethelyn, Wellesley College, Wellesley, Mass.
Irwin, James M., Dartmouth College, Hanover, N. H.
Leighton, Helen, 68 Park Avenue, Canandaigua, New York.
Roberts, Edith A., Mount Holyoke College, South Hadley, Mass.
Rutherford, Elizabeth E., 624 Fullerton Parkway, Chicago, Ill.
Spargo, Mildred W., 5050 Maple Avenue, St. Louis, Mo.
Wallburg, Amy B., 54 Dale Street, Roxbury, Mass.
Williston, Ruth, 297 Crown Street, New Haven, Conn.

3. TABULAR VIEW OF ATTENDANCE

	1907	1908	1909	1910
INVESTIGATORS—Total	60	52	66	62
Occupying Rooms				
Zoölogy	32	32	39	33
Physiology	8	6	7	9
Botany	10	8	4	9
Occupying Tables				
Zoölogy	9	6	14	5
Physiology	1		2	2
Botany				1
STUDENTS—Total	47	48	63	64
Zoölogy	34	19	36	31
Embryology		15	12	10
Physiology	4	3	9	5
Botany	9	11	6	17
UNIVERSITIES AND COLLEGES REPRESENTED	47	51	47	49
By Investigators	26	25	27	26
By Students	21	26	20	24

4. SUBSCRIBING INSTITUTIONS, 1910

Academy of Natural Sciences, Philadelphia.
Columbia University.
Dartmouth College.
Goucher College.
Lucretia Crocker Scholarship, Boston Public Schools.
Massachusetts Agricultural College.
Mount Holyoke College.
Northwestern University.
Oberlin College.
Princeton University.
Rockefeller Institute for Medical Research.
Smith College.
Syracuse University.
University of Chicago.
University of Illinois.
University of Kansas Woman's Table Supported by Mrs. Robinson.
University of Michigan.
University of Pennsylvania.
University of Rochester.
Vassar College.
Washington University Alumni Association.
Wistar Institute of Anatomy and Biology.
Wellesley College.
Yale University.

5. EVENING LECTURES, 1910

Jacques Loeb	"How Does the Spermatozoön Cause the Development of the Egg?"	July 1.
C. Bowyer Vaux	"How to Prepare a Paper for Publication"	July 5.
John B. Smith	"Mosquitoes and Mosquito Campaigns"	July 8.
H. H. Donaldson	"The Percentage of Water in the Central Nervous System of Vertebrates"	July 12.
W. J. V. Osterhout	"Some Aspects of the Action of Mineral Salts upon Plants"	July 15.
E. B. Wilson	"Heredity and the Chromosomes"	July 19.
B. M. Duggar	"The Relation of Plants to Toxic Agents, with Special Reference to the Modification of Toxicity by Chemical and Physical Agents"	July 26.
R. M. Yerkes	"The Relation of Psychology to Biology"	July 29.
J. T. Patterson	"The Questions of Specific Polyembryony and the Determination of Sex in the Armadillo"	Aug. 4.
Ralph B. Perry	"When is a Thing Explained for the Purpose of Science"	Aug 5.
Raymond Pearl	"The Inheritance of Fecundity in the Domestic Fowl"	Aug. 9.

6. MEMBERS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. Life Members

Allis, Mr. Edward Phelps, Jr., Palais Carnoles, Menton, France.

Andrews, Mrs. Gwendolen Foulke, 821 St. Paul St., Baltimore, Md.

Billings, Mr. R. C., 66 Franklin Street, Boston, Mass.

Carey, Mr. Arthur Astor, Fayerweather Street, Boston, Mass.

Clarke, Prof. S. F., Williams College, Williamstown, Mass.

Conklin, Dr. E. G., Princeton University, Princeton, New Jersey.

Crane, Mr. C. R., 2559 Michigan Boulevard, Chicago, Ill.

Davis, Major Henry M., Syracuse, New York.

Endicott, William, Jr., 31 Beacon St., Boston, Mass.

Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.

Farlow, Prof. W. G., Harvard University, Cambridge, Mass.

Fay, Miss S. B., 88 Mt. Vernon Street, Boston, Mass.

Folsom, Miss Amy, 88 Marlborough St., Boston, Mass.

Foot, Miss Katharine, 80 Madison Avenue, New York City.

Gardiner, Miss Eugenia, 15 West Cedar Street, Boston, Mass.

Hammond, Mr. G. W., Hotel Hamilton, Boston, Mass.

Hannaman, Mr. Charles E., 103 First Street, Troy, New York.

Harrison, Provost C. C., University of Pennsylvania, Philadelphia, Pa.

Jackson, Miss M. C., 88 Marlborough Street, Boston, Mass.

Jackson, Mr. Charles C., 24 Congress Street, Boston, Mass.

Kennedy, Mr. George G., 284 Warren Street, Roxbury, Mass.

Kidder, Mr. C. G., 27 William Street, New York City.

Kidder, Mr. Nathaniel T., Milton, Mass.

King, Mr. Charles A.

Lee, Mrs. Frederic S., 279 Madison Avenue, New York City.

Lowell, Mr. A. Lawrence, 171 Marlborough Street, Boston, Mass.

Mason, Miss E. F., 1 Walnut Street, Boston, Mass.

Mason, Miss Ida M., 1 Walnut Street, Boston, Mass.

Means, Mr. James Howard, 196 Beacon Street, Boston, Mass.

Merriman, Mrs. Daniel, Worcester, Mass.

Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.

Minns, Mr. Thomas, 14 Louisburg Square, Boston, Mass.

Minot, Dr. Charles S., Harvard Medical School, Boston, Mass.

Mixter, Miss M. C., 241 Marlborough Street, Boston, Mass.

Morgan, Mr. J. Pierpont, Jr., Wall and Broad Streets, New York City.

Morgan, Prof. T. H., Columbia University, New York City.

Morgan, Mrs. T. H., New York City.

Norcross, Miss Laura, 9 Commonwealth Avenue, Boston, Mass.

Noyes, Miss Eva J., 28 South Willow Street, Montclair, N. J.

Nunn, Mr. Lucian L., Telluride, Colo.

Osborn, Prof. Henry F., American Museum of Natural History, New York City.

Pell, Mr. Alfred, Highland Falls, Orange County, N. J.

Phillips, Dr. John C., 299 Berkley Street, Boston, Mass.

Phillips, Mrs. John C., 299 Berkley Street, Boston, Mass.

Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.

Pulsifer, Mr. W. H., Newton Center, Mass.

Rogers, Miss A. P., 5 Joy Street, Boston, Mass.

Rogers, Mrs. William B., 117 Marlborough Street, Boston, Mass.

Sears, Dr. Henry F., 420 Beacon Street, Boston, Mass.

Shedd, Mr. E. A.

Smith, Mrs. C. C., 286 Marlborough Street, Boston, Mass.

Strobell, Miss E. C., 80 Madison Avenue, New York City.

Thorndike, Dr. Edward L., Teachers' College, Columbia University, New York City.

Trelease, Prof. William, Missouri Botanical Gardens, St. Louis, Mo.

Ware, Miss Mary L., 41 Brimmer Street, Boston, Mass.

Warren, Mrs. S. D., 67 Mt. Vernon Street, Boston, Mass.

Whitney, Mr. Henry M., Brookline, Mass.
Willcox, Miss Mary A., Wellesley College, Wellesley, Mass.
Wilmath, Mrs. H. D., Elliott Street, Jamaica Plain, Mass.
Williams, Mrs. Anna P., 505 Beacon Street, Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City.
Wilson Prof. W. P., Philadelphia Museum, Philadelphia, Pa.

II. Members, August 9, 1910

Abbott, Margaret B., 413 W. Front St., Plainfield, N. J.
Adams, C. F., University of Arkansas, Fayetteville, Arkansas.
Addison, W. H. F., University of Pennsylvania, Philadelphia, Pa.
Alsburg, Carl S., U. S. Dept. Agriculture, Washington, D. C.
Baker, E. H., 5444 Catherine St., Philadelphia, Pa.
Bardeen, C. R., University of Wisconsin, Madison, Wis.
Beckwith, Cora J., Vassar College, Poughkeepsie, New York.
Bigelow, Maurice A., Teachers College, New York City.
Bigelow, Robert P., Massachusetts Institute of Technology, Boston, Mass.
Blatchford, E. W., 1111 LaSalle Ave., Chicago, Ill.
Brookover, Charles, Buchtel College, Akron, Ohio.
Browne, Ethel N., Bennett School, Millbrook, New York.
Buckingham, Edith N., 342 Marlborough St., Boston, Mass.
Budington, Robert A., Oberlin College, Oberlin, Ohio.
Bumpus, H. C., University of Wisconsin, Madison, Wis.
Byrnes, Esther F., 193 Jefferson Ave., Brooklyn, New York.
Calkins, Gary N., Columbia University, New York City.
Calvert, Philip P., University of Pennsylvania, Philadelphia, Pa.
Carlson, A. J., University of Chicago, Chicago, Ill.
Cary, L. R., Princeton University, Princeton, N. J.
Cattell, J. McKeen, Garrison-on-Hudson, New York.
Chester, Webster, Colby College, Waterville, Maine.
Chidester, Floyd E., Clark University, Worcester, Mass.
Child, C. M., University of Chicago, Chicago, Ill.
Clapp, Cornelia M., Mount Holyoke College, South Hadley, Mass.

Clark, Eliot R., Johns Hopkins University, Baltimore, Md.
Coe, W. R., Yale University, New Haven, Conn.
Colton, H. S., 3409 Powellton Ave., Philadelphia, Pa.
Comstock, J. H., Cornell University, Ithaca, New York.
Coolidge, Charles A., Ames Building, Boston, Mass.
Curtis, W. C., University of Missouri, Columbia, Mo.
Dimon, Abigail C., 367 Genesee St., Utica, N. Y.
Dodds, G. S., St. Louis University Medical School, St. Louis, Mo.
Donaldson, H. H., Wistar Institute of Anatomy and Biology, Philadelphia.
Dorrance, Ann, Dorranceton, Pa.
Dorrance, Frances, Dorranceton, Pa.
Drew, Gilman A., University of Maine, Orono, Maine.
Eaton, E. H., Hobart College, Geneva, N. Y.
Eigenmann, Carl H., University of Indiana, Bloomington, Ind.
Field, Irving A., Western Maryland College, Westminster, Md.
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THE SEX RATIO IN HYBRID RATS.

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It has frequently been stated that the sex ratio in mammals can be altered by hybridizing, although there are but few series of observations that give support to such a view. Buffon (1709–1788) seems to have been one of the first to note the apparent excess of males among hybrid offspring; but as his records comprise only a comparatively small number of cases they cannot be considered to afford conclusive evidence that hybridizing changes the normal proportion of the sexes.

In recent times little attention has been paid to the question of the sex ratio in hybrid offspring. Davenport ('06) ascertained the sex of 377 out of a total of 950 hybrid fowls obtained in an extensive series of investigations on inheritance in poultry, and found 204 males and 173 females. Taking the sex ratio for any given lot of individuals as the number of males to each 100 females, it is found that among these hybrids the sexes exist in the ratio of 117.91 males to 100 females. This sex ratio seems to indicate that there is a pronounced excess of males among hybrid fowls, yet Davenport states that the proportion of the sexes in hybrids is normal, and that "the exceptions to the law of equality of sexes in hybrid offspring are individual and not of general significance." Davenport attributes the excess of males among his 377 hybrid fowls to a difference in the death rate of the two sexes, yet he gives no figures to show that among young poultry more females die than do males. Human statistics, as well as the records I have been collecting for the albino rat, indicate that mortality is greater among young males than among young females. Davenport's conclusion does not appear to apply to hybrid birds in general, as Guyer ('03, '09) found a great excess of males among hybrid pigeons, and the data which he has collected regarding the sex of other hybrid birds show a very much greater number of males than of females in practically every case.

In order to study the color combinations in hybrid mice von Guaita ('98, '00) crossed albino mice with Japanese waltzing mice and inbred their descendants through six generations. In his various tables von Guaita gives the sex of the great majority of the hybrid offspring obtained in each generation, yet he makes no comment whatever on the relative proportion of the sexes in these individuals. For the purpose of comparing the sex ratio in hybrid mice with that in hybrid rats, von Guaita's sex records have been collected and the sex ratios calculated for the individuals in each generation. The data thus obtained are shown in the following table.

Table I.

Sex Data for Hybrid Mice (von Guaita).

Generation.	No. Hybrids.	Sex Unknown.	Males.	Females.	No. Males to 100 Females.
F_1	31	3	16	12	133.33
F_2	45	0	21	24	87.50
F_3	77	0	39	38	102.63
F_4	134	21	60	53	113.20
F_5	95	9	48	38	126.31
F_6	25	18	5	2	
	407	51	189	167	113.17

According to these records von Guaita obtained a total of 407 hybrid mice; but he gives the sex of only 356 individuals, of which 189 were males and 167 were females. In this lot of hybrids, therefore, there are 113.17 males to 100 females. This sex ratio is undoubtedly higher than that which is normal for either of the two species with which the experiments began. Schultze ('03) found a nearly equal proportion of the sexes in over 1,000 albino mice that had been bred under different environmental conditions; a similar relation between the sexes existed in the 135 waltzing mice bred by Yerkes ('07).

Raymond and Maud D. Pearl ('08) have tabulated over two hundred thousand records of legitimate births occurring in the city of Buénos Ayres during the years 1896–1905 inclusive, in order to ascertain whether there is a tendency towards an excessive production of offspring of one sex in cross as compared with pure matings. Their tables show that in pure matings, either among Argentine, Italian or Spanish stock, the number of males to each 100 females ranges from 100.77 to 105.55 in various cases. Among these races, therefore, the same relation of the sexes exist as is normally found when any large number of human births are examined. When the parents were of different racial stock there is a marked increase in the relative number of males; the sex ratio showing from 105.72 to 106.74 males for each 100 females. The general conclusion reached by these investigators is that "there is a definite tendency towards an excessive production of male offspring in cross as compared with pure matings in the data considered. Further, it appears that this tendency is uniformly exhibited in all the matings."

The records given above comprise all the sex data for considerable numbers of hybrid offspring that I have been able to find. All of these records are in complete accord, since in each case there is an excess of males that is much greater than that which is probably normal for the parent stock.

For several years past Dr. S. Hatai, of the Wistar Institute, has been crossing the wild Norway rat (*Mus norvegicus*) with the albino rat (*Mus norvegicus albinus*) and breeding their descendants, in order to obtain material for a study of the central nervous system in hybrid rats. The records for the many hybrid offspring that have been examined include the sex data, and Dr. Hatai has generously offered me the use of these records for the study of the sex ratios that is given in the present paper. As far as I am aware, there are no published statistics regarding the proportion of the sexes among hybrid rats, although three investigators, von Fischer ('74), Crampe ('84) and Bos ('94), have carried on extensive hybridizing and inbreeding experiments with these animals.

Cuénot ('99) examined 30 litters of young albino rats containing a total of 255 individuals, and found among them a sex ratio of 105.6 males to 100 females. Records which I have made of the sex of 452 young albino rats belonging to 80 litters give a sex ratio of 107.33 males to 100 females. The sex ratio in the albino rat, therefore, agrees with that for man and various other mammals, since the number of the males is slightly greater than that of the females.

No statistics have as yet been collected regarding the normal proportion of the sexes among wild Norway rats. Judging from the sex ratio found in general among the mammals, it is very probable that there is approximately an equality of the sexes in the wild Norway rat as in the albino rat. In the total of 607 albino rats examined by Cuénot and by myself there was found a sex ratio of 106.46 males to 100 females. This sex ratio is considered, throughout this paper, to express the probable normal relation between the sexes in the wild rat as well as in the albino rat.

Four series of experiments were made in which albino rats were crossed with wild Norway rats. Records are available for a total of 163 individuals belonging to the F_1 generation, of which 89 were males and 74 were females. Among these hybrids, therefore, there is a sex ratio of 120.26 males to 100 females. Although this sex ratio is much higher than that which is probably normal for either of the parent species, it is not as high as that found in von Guaita's first generation of hybrid mice where the sexes existed in the ratio of 133.33 males to 100 females (Table I.).

All of the hybrid rats belonging to the F_1 generation for which records were made were at least five months old when they were killed and examined; the few individuals that died before reaching maturity were not included in the records. It cannot be determined with any degree of certainty, therefore, to what proportion of the entire number of offspring belonging to the F_1 generation the above sex ratio applies. Very few of the young rats died, as far as is known, and there is no reason to suppose that the mortality was greater among the young females than among the young males. It seems probable, therefore, that the sex ratio in the 163 individuals for which data are available is fairly representative for the entire number of offspring produced in the course of the various experiments to which they belonged.

Two of the four series of experiments mentioned above were extended by mating various individuals belonging to the first generation of hybrids. In pairing these animals no attention was paid to their blood relationship, and undoubtedly nearly related individuals were mated in many cases.

In one series of experiments only a very small number of individuals belonging to the second generation of hybrids died before reaching maturity, and the 114 rats for which records are at hand form a very great majority of all of the offspring produced. Of these individuals 61 were males and 53 were females; this gives a sex ratio of 115.09 males to 100 females.

In the other series of experiments the majority of the individuals belonging to the F_2 generation died while immature, and records were made for only 27 individuals. Since twice as many females as males reached maturity, it is evident that in this instance either the mortality was much greater among the young males than among the young females or that there was a very unequal distribution of the sexes in the newborn rats.

Individuals from only one lot of hybrids belonging to the second generation were mated. The animals were paired according to color, their possible blood relationship being entirely disregarded. Dr. Hatai found, as have other investigators who have bred hybrid rodents, that there is increasing infertility among individuals belonging to succeeding generations of hybrids. The total number of hybrid offspring belonging to the F_3 generation was relatively small: many of the rats were stunted in their development, and the majority of them died before reaching maturity. Since records are available for only 23 of these individuals the sex ratio among them can give no idea of the probable ratio in a large number of hybrids belonging to the third generation.

Since in mating individuals belonging to the first and to the second generation of hybrids no attention was paid to their blood relationship, it is very probable that in some cases closely related individuals were paired. There is the possibility, therefore, that inbreeding might have had some influence on the sex of the descendants. According to Düsing ('84), a noticeable increase in the number of male offspring is produced by inbreeding, in man as well as in various mammals. Inbreeding could have had little, if any, influence on the sex ratios in these various lots of hybrid rats. The sex ratio found among the 114 hybrids of the F_2 generation which comprise practically all of the offspring produced in the series of experiments to which they belonged, is somewhat less than that found among the offspring produced by crossing pure stock (Table II.). The data for the other individuals belonging to the F_2 generation, as well as those for the hybrids of the F_3 generation, show an excess of females. These latter records cannot be considered to furnish evidence against Düsing's theory, since they include such a very small number of individuals. There seem to be no records, except those given by Düsing, that show that inbreeding alone produces a relatively greater number of male than of female offspring. Schultze was unable to detect any change in the sex ratio of albino mice that had been inbred for four generations, and I have found no unusual excess of males among some 500 individuals belonging to five generations of inbred albino rats.

Records are available for 121 other hybrid rats, most of which were the offspring of reciprocal crosses. These individuals all belonged to the F_2 or to the F_3 generation of hybrids; and among them were 72 males and 49 females. These hybrids were selected, because of their size and condition, from a considerable number of individuals for which no records were made. There was, therefore, undoubtedly an unconscious selection of males in choosing the rats to be examined, since the adult male rat is considerably larger and heavier than the female. The data for these various lots of selected individuals have been combined in one group, since they are of value only because they show the sex of a considerable number of hybrids.

The following table gives a summary of the sex records for all of the hybrid rats recorded.

TABLE II.

SEX DATA FOR HYBRID RATS.

Generation.	No. Hybrids.	Males.	Females.	No. Males to 100 Females.
F_1	163	89	74	120.26
F_2	114	61	53	115.09
F_3	27	9	18	50.00
F_2 and F_3	121	72	49	146.93
	425	231	194	119.07

In the total of 425 hybrid rats for which records were made, 231 were males and 194 were females. Among these individuals, therefore, there is found a sex ratio which is considerably higher than that which is probably normal for either the albino or the wild Norway rat, since there are 119.09 males to 100 females.

If from the above table we omit the records for the 27 individuals belonging to the F_2 generation of hybrids which formed only a very small percentage of the total number of offspring produced in the experiment to which they belonged and also the records for the selected individuals belonging to the F_2 and to the F_3 generation, there remain the data for 277 individuals which comprise the very great majority of the hybrid offspring obtained in several series of experiments. It would seem as if the sex ratio in these individuals might justly be taken to represent the probable sex ratio in any large lot of hybrid rats. Of these individuals 150 were males and 127 were females; this gives a sex ratio of 118.11 males to 100 females. This sex ratio is but very little lower than that found in the total number of hybrids for which records are at hand, and it is not very much higher than that in the 356 hybrid mice bred by von Guaita (Table I.).

The excess of males among these hybrid rats is seemingly beyond the limits of normal variation in the proportion of the sexes in the pure stock, and it is too uniform in the various series of experiments to be attributed to chance. It appears, therefore, that hybridizing alters the sex ratio by producing a marked increase in the relative proportion of males. This conclusion is in essential agreement with that reached by Buffon, by R. and M. Pearl and by Guyer.

Guyer ('09) has offered an explanation for the excess of males among hybrid offspring which accords with the theory, advocated by a number of investigators, that sex is determined in the ovary chiefly by nutritive conditions. Guyer suggests that in the zygote produced by cross fertilization there would probably be "more or less default in the metabolic processes because of the incompatibilities which must necessarily exist between two germplasms so dissimilar." An interference with the metabolic processes would naturally retard the constructive phases of metabolism in the fertilized ovum, and therefore tend to the production of relatively more males, since the theory assumes that females are produced only when the conditions are most favorable for constructive metabolism.

To explain the sex ratio in hybrids according to the current hypothesis that the male is the sex-determining factor seems to necessitate the further assumption that fertilization is selective when individuals belonging to different races are crossed, the egg offering greater resistance to the entrance of a spermatozoan that is female-producing than to one that is male-producing. There is little evidence, as yet, that fertilization is ever selective. The probability of its occurrence as a normal phenomenon has recently been advocated by Heape ('09), although Wilson ('10) considers it "so improbable as almost to invalidate any interpretation into which it enters."

Guyer ('03) has shown that there is considerable amount of degeneration in the testes of many hybrid pigeons; abnormal mitoses and misshapen spermatozoa being of frequent occurrence. Other observers have stated that the gonads in hybrids are more or less defective; but none of them have made a histological investigation in order to asertain what structural changes have been produced. Guyer's observations strongly suggest that results of value may be obtained by a study of the gonads in other hybrids. Material is being collected for an investigation of the gonads in hybrid rats, in the hope that it will at least give some clue to the cause for the increased sterility in succeeding generations of hybrids, even if it affords no evidence that will enable one to offer a satisfactory explanation for the altered sex ratio in hybrid forms.

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THE RHYTHMICAL MOVEMENTS OF LITORINA LITOREA SYNCHRONOUS WITH OCEAN TIDES.[1]

J. D. HASEMAN.

INTRODUCTION.

Through the kindness of Dr. Sumner, the writer was given an opportunity to work in the laboratory of the Bureau of Fisheries at Woods Hole, Mass., during the summer of 1907. He is indebted to Dr. Sumner for many valuable suggestions.

The purpose of the present paper is to show that the movements of *Litorina litorea*, which are synchronous with ocean tides, are not due, directly, to either geotropism or phototaxis but to the action of the film of water on the more or less exposed snails. In arriving at this conclusion many experiments were performed not only in the laboratory but also under natural conditions. In addition, the exact habitats of three species of *Litorina* were studied in detail. Such observations are necessary when the natural oscillations of *Litorina*, which correspond to the rise and the fall of the tides, are to be considered.

Lastly the writer will attempt to show that *Litorina litorea* does not retain a rhythm, which is synchronous with the tides, either in quiet aquaria or in nature when the effects of films of water have been removed by keeping the snails permanently submerged.

I. THE HABITAT OF *Litorina*.

(*a*) Individuals of *Litorina rudis* are as a rule found on rocks which lie between the tidal marks, but occasionally an individual occurs on rock weeds and even on the muddy shore. This species was abundant everywhere in the region of Woods Hole, excepting in Grape and Tashmo ponds, both of which are brackish.

(*b*) Individuals of *Litorina palliata* live as a rule on rock weeds which grow between the tidal marks. This species was not encountered at Nobska Point or in Grape, Lagoon and Tashmo ponds. It is always more abundant along protected coasts and hence its absence from Nobska Point, which is exposed to the open sea, was to be expected.

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[1] The final preparation of this paper has been delayed on account of the writer's long trip in South America for the Carnegie Museum. Only the more important results are stated and many of the details have been omitted.

(*c*) Individuals of *Litorina litorea* (said to have been imported to the American coast from Europe) usually live on rocky surfaces which lie between the high-tide mark and one foot below the low-tide mark. The species was not found in Tashmo Pond but a few young individuals were seen in Grape Pond living on transplanted oysters. This observation, taken in connection with others described below, indicates that the absence of *Litorina* from these brackish ponds was not due, directly, to the lower specific gravity that exists there.

In order to explain the restricted distribution of *Litorina* the following observations and experiments were made:

A. *Food.—Litorina palliata* is nearly always found on two common species of rock weeds, and its supply of food is doubtless associated with these plants, while *Litorina rudis* and *litorea* eat small green algæ which grow more abundantly on the rocks which are situated between the tidal marks.

When individuals of *Litorina litorea* were placed in shallow aquaria, which contained rocks from the sea shore, from Lagoon Pond or from the street and contained also pieces of glass and fish they came to nest for the most part on the rocks taken from normal sea water. The snails, which were taken from the entrance to Lagoon Pond with their accompanying rocks, also settled on the sea rocks.

B. *Salinity.*—When individuals of *Litorina litorea* were placed in a mixture of equal parts of fresh and sea water, they were able to crawl about; but when they were placed into a mixture of two parts fresh and one part sea water, all died within eight days. When they were placed in sea water, to which as much as fourteen grams of sun evaporated sea salt had been added to each 800 c.c. of sea water, all died within eight days. The specific gravity of the various test solutions indicated that *Litorina* could live in water which has a lower specific gravity than was observed in Grape and Tashmo ponds.

C. *Temperature.*—The variation in the temperature along the coast did not appear to affect the distribution of *Litorina*. Snails were subjected to much greater changes of temperature than occur along the coast and no observable effects were detected.

D. *Pressure.*—Several individuals of *Litorina litorea* were submerged in cages, which contained sea shore rocks, to a depth of 8, 18 and 30 feet of water. Two weeks later the snails appeared to be perfectly normal.

E. *Aeration.*—*Litorina litorea* is very sensitive to slight changes in aëration and consequently it is very difficult to arrange the condition in an aquarium so that they behave normally.

F. No direct effects of light on the distribution of *Litorina* could be detected either by experiment or direct observations made in nature.

G. *Waves.*—When individuals of *Litorina litorea* are violently splashed by waves, they become quiescent.

H. *Character of the Surface.*—Individuals of *Litorina litorea* are rarely found on either sand or mud, due in part to the absence of food in such places and in part to the difficulty encountered in crawling over such surfaces. This is especially true when the waves constantly change the position of the same and hence tumble the snails about at random.

I. *Moisture.*—*Litorina litorea* does not crawl on dry surfaces.

II. Factors which Determine the Movements of *Litorina.*

It appears evident from the preceding data that the character of the surface, moisture and food are the chief factors which determine the litoral distribution of these snails. But as I hope to demonstrate in the following pages, these three factors produce random movements of the snails and should not be confounded with the factors which directly produce rhythmical movements corresponding to the rise and fall of the tides.

The following experiments and observations will, I think, determine what the "directive force" of rhythmical movements is.[1]

1. The snails which are located on flat horizontal surfaces between the tidal marks do not show rhythmical movements which correspond to those of the tide. The snails which are located or placed below the low-tide mark on either a vertical or a horizontal surface do not show rhythmical movements.

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[1] The majority of the experiments are easily performed along the seashore by marking the snails and the stones.

But the snails which are located on any more or less vertical surface between the tidal marks do exhibit rhythmical movements which correspond to those of the tides.

2. When a stone, which has snails crawling on a flat surface below the low-tide mark, is raised and lowered in the sea, the snails do not show rhythmical movements which correspond with those of the stone, *i. e.*, their movements were not directive. The same results are obtained when, with the flat surface horizontal, the stone is raised out of and lowered into the sea. But when, with the surface vertical, the stone is raised out of and lowered into the sea, the snails at once showed rhythmical movements which correspond to those of the stone. These same snails were known (from observations made on snails placed between the tidal marks and below the low-tide mark) to have shown in some cases and not to have shown in other cases rhythmical movements during several days previous to the experiments. Therefore any alleged retained rhythm has nothing to do with the above results.

Even more interesting is the fact that when, with a vertical surface, a stone, upon which snails were crawling at random, was raised out of the sea, the snails always followed the vanishing film of water even when the vertical surface was rotated through an angle of 180°. In this case the rotation of the vertical surface would reverse the direction of motion of the film of water and the snails would at once turn around and follow it. But if most of the water was previously removed from the surface of the stone, in order that the film might entirely disappear before the snails (which were crawling downward in the direction of the vanishing film) had reached the lower surface, and if, as the film was drying up, the vertical surface was rotated through an angle of 180°, the snails continued to crawl for some time in the direction in which they had started. In other words, the snails crawled upward instead of downward. They continued to crawl thus until the rough surface, food and moisture either deflected or stopped their movements. In the above experiment, the mere turning of the moist but filmless surface through an angle of 180° does not seem adequate to reverse at once the reaction to gravity and light, if either of these have a direct influence on the rhythmical movements of *Litorina*.

3. When the eyes of *Litorina litorea* are destroyed, they still show rhythmical movements, on vertical surfaces between the tidal marks. Individuals of *Litorina litorea* also oscillate with the tides during dark nights.

4. During the months of July and August, the writer could not detect any relative change in the position of snails on large boulders. If phototaxis is an important factor, a marked change would be expected because the daily lagging of the tides as well as the diurnal and monthly changes of the relative solar position of the earth, constantly change the relative angle of the rays of light.

5. When it rains on exposed snails, they become quiescent just as they do when splashed by the water. Therefore during stormy as compared with fair days, a considerable difference in the amount of oscillations of *Litorina litorea* was noted.

6. Submerged snails could be only slightly or not at all directed by currents of water. The currents were produced artificially and also by moving stones with snails through the water. Hence the behavior of *Litorina* on vertical surface between the tidal marks is not of a rheotropic nature.

7. When individuals are left high and dry on vertical surfaces during low tide, they come to rest "directed upward," *i. e.*, with their head end toward the sky. This is true for all sides of the stones and is obviously due to the shape of the aperture of the shells which makes it far easier for exposed individuals to cling thus to vertical surfaces.

8. Individuals of *Litorina litorea* are on an average found slightly higher on the north side of large boulders than on the south side. This appears to be due to greater moisture and a higher growth of both algæ and barnacles over which snails crawl with difficulty.

9. Some bottles partly filled with water and air plus hydrogen and others partly filled with carbon dioxid were used to test the effect of crawling out of the water into a gaseous medium and vice versa. No differences were detected from that of crawling out of water into the atmosphere unless so much hydrogen and carbon dioxid were used that the snails were asphyxiated.

10. Thin films of olive oil and kerosene were placed on water

in aquaria to test the effect of surface tension. Under these conditions the snails had considerable difficulty in breaking the surface film and always hesitated at the surface pushing themselves to the right and to the left until their bodies were elevated sufficiently to break the surface tension. Their behavior under the above condition showed that the hesitation on entering and leaving the surface was due to the surface film and not to phototaxis, hydrotaxis or chemotaxis.

11. When individuals of *Litorina litorea*, *rudis* and *palliata* were placed in a small round aquarium equal areas of which were painted brown, yellow and dark olive, they as a rule came to rest on the dark olive sector, rarely stopped on the brown sector and still more rarely on the yellow sector. When the same snails were placed in a similar unpainted aquarium before a window, about one half of them came to rest on the lighter side and half on the darker side. The position of the aquaria were interchanged and the same results were obtained. Various shading experiments were tried and neither positive or negative phototaxis could be detected. In nature one sees snails crawling at random from lighter areas into shaded areas and vice versa.

The writer was unable to detect why the snails stopped on the dark olive sector, but inasmuch as such conditions are not met on vertical surfaces of stones between the tidal mark, the results appear to have nothing to do with the rhythmical movements of *Litorina*.

12. Many snails were placed in quiet aquaria and aquaria in which running water was kept at a constant level and they never exhibited any signs of oscillations which corresponded to those of the tides. In these experiments, snails which were known to have been previously oscillating with the tides were used. I also placed snails, which had been oscillating with the tides, on vertical surfaces below the low tide mark and an average of many counts did not show rhythmical movements which corresponded to those of the tides.[1] Only when the snails are touched by the surface film of water are directive movements called forth.

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[1] Morse has recently obtained a similar result.

DISCUSSION.

Mitsukuri ('01) found that the roughness of the surface scatters the snails and that they never crawl on dry surfaces. He also stated that *Litorina exigua* hesitated on entering the water and concluded that their oscillations with the tides were due to phototaxis. In this connection the writer found that the snails hesitated as much on leaving the water as they did on entering it. This fact, taken in connection with several of my experiments and especially those on surface tension, show that the observed hesitation was due to the effect of the surface-film of water and not to phototaxis.

Bohn ('05) also concludes that phototaxis is the important factor which determines the oscillatory movements of *Litorina*. He states that *Litorina rudis, obtusata* and *litorea* have for all their lives been desiccated again and again, and when placed in quiet aquaria, all three species retained a period of rhythm synchronous with the tides and lasting from thirteen hours to fourteen days. My observations show that *Litorina litorea* does not have such an established rhythm.

The behavior of *Litorina* in experiment 2 is sufficient to show that the "directive force" in their rhythmical movements synchronous with the tides is the film of water and not phototaxis. Also the fact, that only snails on vertical surfaces between the tidal marks have oscillatory movements, shows that the directive force is not phototaxis because many snails on flat horizontal surfaces could crawl to the edge of the surface and descend to the base of the stone. Especially the observations made during dark nights as well as many other experiments already cited indicate that Bohn's view is untenable.

CONCLUSION.

In conclusion, then, it appears that submerged individuals of *Litorina litorea* crawl about on large stones at random, perhaps in search of food. They never crawl on dry surfaces and consequently are not found above the high-tide mark. When the tide rises, the environment is temporarily enlarged and consequently many submerged snails on more or less vertical surfaces by mere chance will crawl up higher.

The individuals which were previously left high and dry are already directed upward and hence follow the rising tide. Before maximum high tide is reached, the unevenness of the surface along with the presence and absence of food scatter the snails at random because the rapidly rising tide soon submerges them and then there is no "specific directive force."

When the tide begins to fall some of the snails are already crawling downward and those that are not will be directed thus by the surface film. During a falling tide, some snails are crawling down beneath the surface, some with the surface, and some above the surface. As the tide falls lower and lower, many snails lag behind more and more until they are finally left on filmless surfaces under which conditions there is no specific directive force and such snails are left exposed. This lagging appears to be due to feeding, rough surfaces, rough sea, rapid fall of the tide and the more rapid desiccation on certain days. As a result of all these factors the amount of oscillation varies from day to day.

The snails on either flat horizontal surfaces between the tidal marks or on any kind of a surface below the low-tide mark have no "specific directive force" and consequently do not exhibit oscillatory movements synchronous with the ocean tides.

SUMMARY.

1. *Litorina rudis, palliata* and *litorea* are found in definite zones.

2. Individuals of *Litorina litorea*, located on vertical surfaces between the tidal marks, exhibit oscillatory movements which correspond to those of the tides but they do not exhibit such movements when they are located either on flat horizontal surfaces between the tidal marks or on any kind of a surface below the low-tide mark.

3. The primary directive force for rhythmical movements is the surface film of water. The secondary directive forces are the quiescent position of desiccated individuals, character of the surfaces, moisture and food.

4. *Litorina litorea* has no established rhythm in the absence of tidal changes.

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3. **Morse, Max Withrow**
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THE LETHAL EFFECT OF PURE DISTILLED WATER ON THE VINEGAR EEL (ANGUILLULA ACETI).[1]

JAMES FRANCIS ABBOTT AND ETHEL LEIGH RICHARDS.

The question of the toxicity of distilled water for aquatic organisms appears to be still an open one. An excellent summary of the literature of the subject has been given by Bullot.[2] Ringer first noted that distilled water is toxic for a variety of organisms, and attributed this toxicity to (1) an abstraction from the organism of salts which are necessary for its life, or (2) the penetration of water into the cells through osmosis, or (3) the imbibition of water by the "intercellular substance." About the same time a contrary opinion was advanced by Nägeli[3] who claimed that it is not the purity of the water but the presence of the slightest trace of copper (even so small a quantity as 1/77,000,000) that gives water its toxic qualities, whereas if water of this sort is redistilled in glass it loses its toxicity. Shortly after this, Locke questioned Ringer's conclusions and decided that the latter's results too were due to the presence of copper rather than the toxicity of the water and in 1895[4] confirmed his belief by experiments with the tadpole and *Tubifex*. Ringer[5] then retested his experiments and partially recalled his former conclusions, agreeing with Locke that "pure water (*i. e.*, that distilled in glass) is completely harmless for the animals in question. This was confirmed by Jennings for *Paramecium*, Miss A. Moore for trout and tadpole and F. R. Lillie for *Planaria*. On the other hand Lyon[6] investigated the action of distilled water on developing *Arbacia* larvæ and found that artificial sea water made up of tap water or ordinary distilled water, whether vaporized in copper or glass, is very toxic for these larvæ although tap water which has been boiled long enough to concentrate it one third is much less toxic than ordinary distilled water. For this and other reasons he concluded that the chief toxic agent in such waters is the ammonia which they hold in solution. In fact he found that the larvæ often developed better in ammonia-free artificial sea water than in normal sea water, a result that may be due, as he suggests, to the presence of ammonia in natural sea water.

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[1] From the Zoölogical Laboratory, Washington University, St. Louis, Mo.

[2] Bullot, *Univ. of Calif. Pub., Physiol.*, I., 1904, 199.

[3] Nägeli, *Denksch. d. Schweiz. Naturforsch. Gesells.*, Bd. 33, 1893.

[4] Locke, *Jour. Phys.*, 18, 1895, 319.

[5] Ringer, *Jour. Phys.*, 22, 1897.

[6] Lyon, BIOL. BULL., 6, 1904, 198.

About the same time Bullot[1] published a thorough investigation of the toxicity of distilled water for the fresh water *Gammarus* and found that the water distilled over glass with all the precaution employed in a physical chemistry laboratory was toxic for this form although to a less extent than water distilled over copper. He found, however, that the presence of NaCl in such a dilution as is expressed by a concentration of $0.00008N$ enables the *Gammarus* to live indefinitely in such a medium. Loeb[2] suggests that in such a case, "The presence of a trace of NaCl in the distilled water possibly preserves the membrane better or maintains better the secretory activity of the cells, so that the animal can be freed from the excess of water which diffuses into it."

The group of Nematodes is noted for the extraordinary resistance which the cuticle offers to external media. The common vinegar or paste eel, *Anguillula aceti*, is well known to occur normally in weak vinegar, although acetic acid is to most organisms a rapidly acting poison. *Anguillula aceti* has been observed in our laboratory to live more than 24 hours in Tellyesnicky's fixing fluid and three or four hours in Gilson's fluid. The cuticle of this worm might therefore be expected to be quite unaffected by such ions as ordinary distilled water may contain. It is of interest to note in this connection that Devaine[3] in his biological investigation of the vinegar eel observed that the worms live at most but eight days in distilled water. This effect might be due to the lack of the acetic acid which ordinarily forms a part of their normal environment or to the direct effect of the water itself. It was soon found that although the worms live best in an environment of weak vinegar, it is the sugar rather than the acetic acid which appears to be the essential element of the medium.

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[1] Bullot, *l. c.*

[2] Loeb, J., "Dynamics of Living Matter," p. 50, 1906.

[3] *Compt. Rend.*, T. 61, 1865, p. 259.

With the hope of analysing the relations of this organism to its environment, a number of lines of investigation have been taken up in connection with the reactions of the nematodes to various media and to different food substances. The present paper deals only with the effect produced on the worms by distilled water. The worms used in these experiments were found in rather stale weak vinegar of an acidity of 8/10 *N*. No difficulty was experienced in breeding them abundantly in the laboratory both in this medium and in cider. Two kinds of distilled water were employed. One of these was such as is used in the ordinary work of the laboratory. This was distilled in an automatic copper still and will be designated "A." The second water, which will be designated "B," was obtained by distilling the first kind over potassium dichromate and sulphuric acid, condensing in block tin out of contact with the air and redistilling over barium hydrate. The first six tenths of this distillate was rejected and the remainder gave an extremely pure sample, CO_2-free and showing no ammonia reaction (Nesler test) even after the lapse of twelve hours. This water when tested in a conductivity apparatus of the department of physical chemistry gave no measurable conductivity with a resistance of 20,000 ohms and may be considered to be practically free from electrolytes. It was kept in well steamed, hard glass bottles. In each experiment every precaution was taken to remove all traces of the medium in which the worms were living. The worms were washed 6 to 8 times with distilled water in a centrifuge, and in the cases where the distilled water "B" was used, two or three times more in this. They were then allowed to remain in the "B" water for about 24 hours to insure a thorough rinsing. Finally they were again washed in pure water previous to their insertion in the new medium. The bottles and test tubes used to contain the worms in the first two sets of experiments were of ordinary soft glass and in the latter ones, *Schott und Genossen*, Jena resistance glass was employed. These containers were thoroughly soaked in chromic acid, and previous to use were repeatedly rinsed in distilled water. They were then steamed for a greater or less length of time under pressure of 150 pounds. An attempt was made to put as nearly as possible the same number of worms in each culture tube but owing to their minute size it was of course impossible to do more than approximate the number.

Contrary to expectations the worms lived much longer in the ordinary laboratory water "A," presumably full of copper and ammonia than in that distilled over potassium dichromate and free from CO_2, NH_3, copper and other ions. In "A" and soft glass tubes they lived 31 days. In "B" water and soft tubes they lived sixteen days and in "B" water and Jena insoluble tubes they lived but six days. These results would seem to indicate that the presence of the electrolytes in ordinary distilled water and of the ions dissolved from the glass prolonged the life of the worms. Because of the fact that the purer the water the greater its solvent power on glass, it is still to be determined whether this effect is due to the substances dissolved from the glass or to the copper and other ions in the distilled water itself. In the series with "B" water and Jena glass, in which the worms lived but six days, there were no impurities in the water and practically no solvents from the glass. So we are forced to conclude that the toxic character of the water itself was the cause of the death of the worms. This is borne out by observations on the behavior of the worms under such conditions. The outer integument of the nematode in contrast to its normal hardness and resistance, appeared to become very viscous after a sojourn in pure water. When any obstacle was encountered in swimming, —the side of the tube or the surface of the water, or other worms, the organisms stuck fast. This alteration of the natural condition of the cuticle was so marked that after a time the worms bunched together in masses, unable to free themselves or else coming in contact with the surface of the water, they hung suspended. This effect would seem to bear out Loeb's suggestion quoted above. That there is a marked taking up of water on the part of the organism seems quite evident. The body becomes swollen and the worms lose their elasticity, becoming more or less rigid through turgor and swimming with the body almost in a straight line without the characteristic twitching of the ends. The worms grow more and more impotent until at last instead of swimming directly towards the surface as is usually the case, they swim in a haphazard manner and in spite of instinctive efforts to reach the top of the water are unable to advance more than an inch or two, falling back towards the bottom with every effort. This may be due to decreased vitality but may also be due to a greater weight on account of the imbibition of water. Repetitions of these experiments have confirmed the results in every instance. Experiments have been undertaken to determine what ions control or effect the permeability of the cuticle to the water.

SUMMARY.

1. *Anguillula aceti* succumbs in six days to very pure water free from electrolytes whereas in ordinary distilled water it will live for much longer periods.

2. The toxic qualities of the pure water appear to reside in its solvent effect on the normally resistant cuticle of the worm which is rendered much more permeable to water.

3. Death is accompanied by physical manifestations suggestive of great imbibition of water by the tissues.

4. The presence of electrolytes in the medium, derived either from solution of the glass culture tube or carried by "impure" samples of distilled water, prolongs the life of the organisms in question, apparently by preserving the character of the membrane so that the tissues will not imbibe water.

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BIOLOGICAL BULLETIN

ECOLOGICAL SUCCESSION.

II. POND FISHES.

VICTOR E. SHELFORD.

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I. INTRODUCTION.

In the first paper of this series ("Stream Fishes and Physiographic Analysis"), we pointed out the reasons for undertaking this investigation and stated the purposes around which the work has centered. There we were concerned with the value of the principles of physiography in a method of locating the animal in the environment and of determining something of its character as a whole. Here we are to discuss the value of plant succession in a similar way and to have a better opportunity to show the validity of the principle of succession as applied to animals. Furthermore, as we pointed out in the other paper, ecological succession is to be differentiated from geological succession. Ecological succession is succession of ecological types regardless of species, while geological succession is the succession of species. The data presented here affords an excellent opportunity to bring out the differences and relations of these two types of succession.

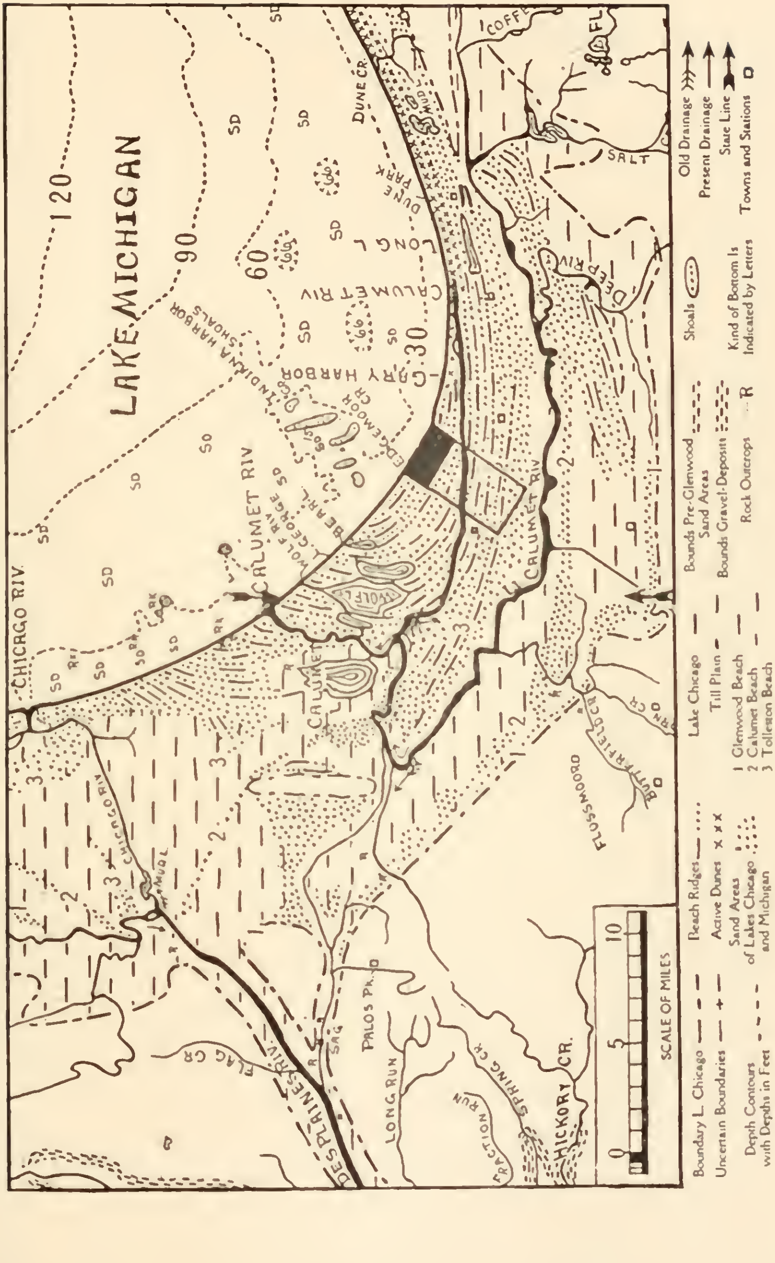
We noted also in the first paper that the first recognition of plant and animal succession came with the development of genetic physiography. It was mainly the successsion which accompanies physiographic change. Cowles ('01) also clearly recognized succession due to the action of plants themselves. This latter idea has been elaborated by Clements ('05) and essentially demonstrated by Schantz ('06) and Dacknowski ('08). Animals must obviously play an important rôle in this type of succession, but unfortunately this has not been investigated.

The succession with which we will deal in this paper is that resulting fron the action of organisms on their own environment. For all practical purposes the area selected for this study has been in a condition of physiographic stability for a considerable period. The selection and analysis of the place of study is the most important step in the whole investigation. Indeed there are only a few suitable localities in North America.

II. Area of Study.

Owing to the fact that succession is always either dependent upon, or modified by changes in conditions, a correct interpretation of this phenomenon depends largely upon accurate knowledge of the area under consideration.

1. *Location and General Character.*—The ponds which are the subject of this study lie in the sand area at the south end of Lake Michigan, within the corporate limits of the city of Gary, Ind. They may be reached from stations known as Pine, Buffington, or Clark Junction. This locality is characterized by a large series of sand ridges, for the most part nearly parallel with the lake shore (Map I.). Their average width is about 100 feet. They are separated by ponds which are somewhat narrower (Map II., p. 131). Most of these ponds are several miles long. They vary in depth during the spring high water, from a few inches to four or five feet. They describe an arc somewhat longer than the lake shore (Map I.), and are farthest from it about midway of their lengths.


LAKE MICHIGAN
120
90
60
30
SD
CHICAGO RIV.
CALUMET RIV
INDIANA HARBOR SHOALS
GARY HARBOR
CALUMET RIV
LONG L
DUNE PARK
DUNE CR.
MUD L.
COFFEE
SALT CR.
DEEP RIV.
EDGEWOOD
WOLF L.
WOLF RIVER
GEORGE
BEAR L.
CALUMET L.
CALUMET RIV
CHICAGO RIV.
MUD L.
DESPLAINES RIV.
FLAG CR.
SAG
PALOS PK.
LONG RUN
SPRING CR.
TRACTION RUN
HICKORY CR.
FLOSSMOOR
BUTTERFIELD CR.
THORN CR.
SCALE OF MILES
0
5
10
Boundary L. Chicago
Uncertain Boundaries
Depth Contours with Depths in Feet
Beach Ridges
Active Dunes x x x
Sand Areas of Lakes Chicago and Michigan
Lake Chicago
Till Plain
1 Glenwood Beach
2 Calumet Beach
3 Tolleston Beach
Bounds Pre-Glenwood Sand Areas
Bounds Gravel-Deposits
Rock Outcrops R
Shoals
Kind of Bottom Is Indicated by Letters
Old Drainage
Present Drainage
State Line
Towns and Stations

MAP. I. This shows the arrangement of deposits at the south end of Lake Michigan. The general direction of the beach ridges is indicated and accordingly the general directions of the ponds. The number of such ponds and ridges cannot be indicated on a map of this scale. The former extent of Lake Chicago and subsequent lakes is indicated. The area in which the fish have been studied is represented by the larger rectangle. The black rectangle represents the area covered by Map. II.

Originally, there were probably a number of outlets to the system of ponds which were joined to one another by these outlets and through various low places in the sand ridges. In the area of our concentrated study there is an outlet (Map II.) which has served to connect all the younger ponds in an intimate fashion.

The building of sewers associated with the growth of northern Indiana towns (Whiting, East Chicago, Hammond and Gary) has drained large portions of the ponds, while roads and railroads have isolated other portions.

2. *Origin of the Ponds.*—During the final retreat of the North American ice sheet, its Lake Michigan lobe stood for a time with its southern end at the crest of the Valparaiso moraine which lives concentrically around the southern end of Lake Michigan. When the ice retreated from this position, water occupied the space between this lobe of the sheet and the moraine. This was Lake Chicago, the forerunner of Lake Michigan. After having stood respectively 60 feet, 40 feet and 20 feet above its present level, long enough to deposit conspicuous beaches, it took up a position at a 12-foot level. The waters appear to have fallen gradually from this level. At present one or two ridges and depressions similar to those found above the water on old Lake Chicago plain, are below the surface of the water along the shore at the south end of Lake Michigan. The retreat of the water has evidently exposed such ridges as fast as they were formed. This has left a *series of parallel ridges and ponds arranged in the order of their age—the oldest farthest from the lake, the youngest nearest the lake.*[1]

These ponds are not all of the same size. The largest ones were selected for study and will be referred to in the paper by number as the entire series is counted inland from the lake shore. There are between seventy-five and ninety of these ponds or depressions between the lake shore and the 20-foot beach level. This is the maximum number. Map I. shows that as we pass in either direction from the area of study their number decreases.

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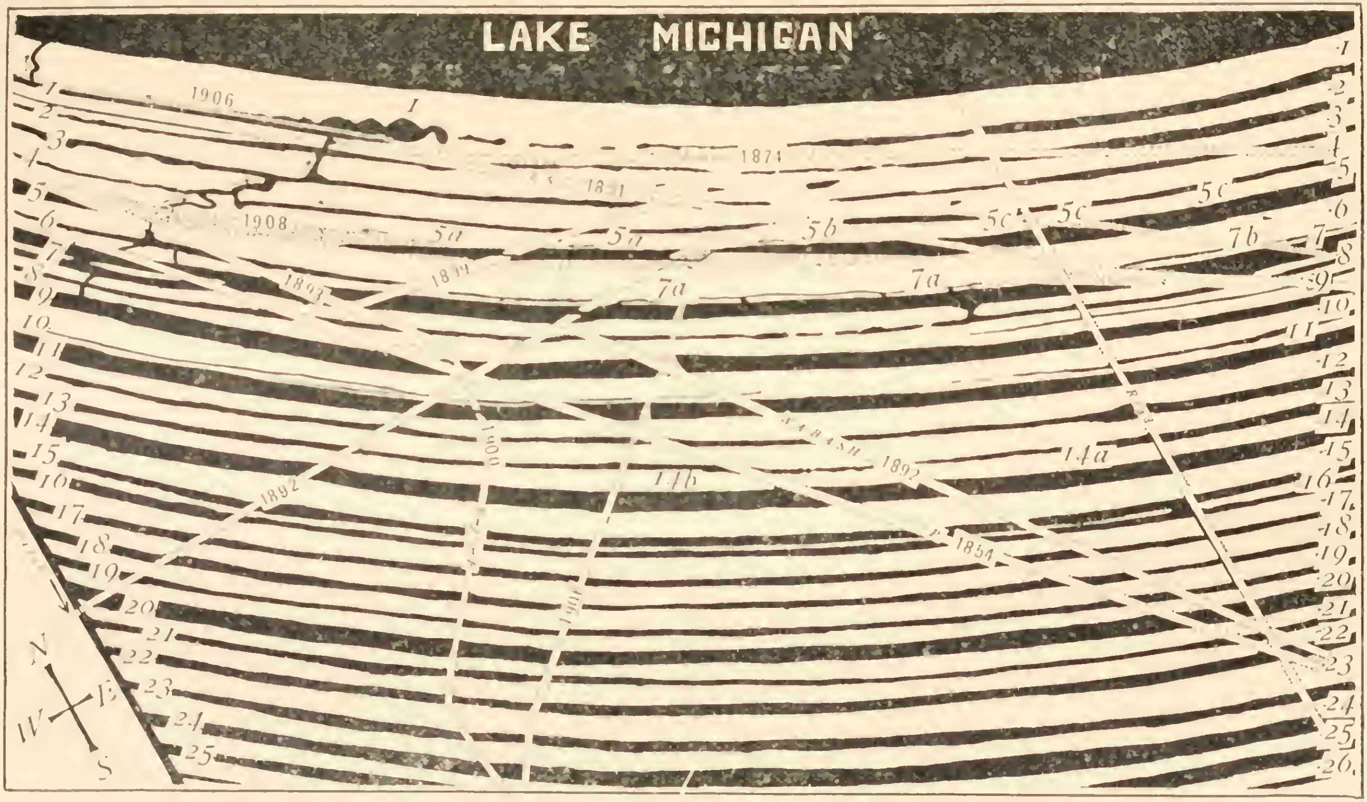
[1] For a treatment of this subject, see articles cited in the bibliography but not specifically referred to here. Professor R. D. Salisbury tells me that there is no question concerning the relative age, from physiographic evidence alone.


LAKE MICHIGAN
1
1906
1874
1881
1908
1893
1899
1892
1854
1900
1901
WABASH
ROAD
5a
5b
5c
7a
7b
11b
14a
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
N
E
W
S

MAP II. Shows the area of special study. The water is shown in black; the ridges and road and railroad grades, in white. The numbers 1, 5, 7, 14, etc., refer to the numbers of the ponds and ridges opposite which they are placed, as these ponds and ridges are counted from the lake shore. The dates associated with the indicated railroad grades refer to the approximate date of building of the grade, and probable damming of the long ponds where sluices were not maintained. The dotted lines are to bound the railroad grades; stippled areas represent recent filling.

Map II. shows the relations of the first twenty-four of these ponds. The recent changes of this smaller area are indicated on this map by the dates of the building of the various road and railroad grades.

III. The Data.

All methods of collecting have been employed. The dip net has been found most effective, but the drought of 1908 and draining by sewers have yielded cruical results in Ponds 1, the outlet, Pond 5*a*, 5*c*, 14*a* and 7*b*.

With the exception of *Amia calva*, the records are for adults, or for young where the pond is known to be *isolated*, and the presence of adults is therefore necessarily implied.

The record of *Ameiurus melas* in Pond 56 is based upon the presence of young and adults; identification of the adults depended upon identification of the young, because the adults were too badly macerated to make identification practicable.

1. *Distribution of the Species of Fish.*—The distribution of the fish in the various isolated parts of the ponds is of much importance. Turning to Map II., we note that the extension of the ponds to the east and west is not shown. Toward the east Ponds 1 to 17 become shallow near the Gary Steel Plant and are *not* connected with other bodies of water. Parts of Ponds 1 to 7 have been directly connected with the lake by the outlet (Map II.) until within the past few years. Excepting in the case of pond 7, other connections with the lake, to the northwest of the area shown on our map, have probably been closed for a much longer period. We may concern ourselves largely with the parts of the ponds to the east of the outlet, which have been isolated by railroad grades, and with their relation to the outlet.

Table I., taken with Map II., presents the exact data on the distribution of the fish in the various parts of the ponds. All fish nomenclature is after Forbes and Richardson, '08.

In Table I. we note first that the large-mouthed black bass, the sunfishes, the pumpkin seed, and the warmouth, are all confined to the outermost pond, or Pond 1. The passage from pond 1 to the outlet and to pond 5 was open until 1906. The study began in 1907. In the autumn of 1908 the drought reduced the water in the outlet to a minimum, but none of these species was found there, though the passage between the outlet and pond 1 had been open two years before. Why have the fish vacated or avoided the outlet? One can only suggest a behavior reaction as the cause.

TABLE I.

Distribution of the Fish.

The letters and numbers at the heads of the columns refer to the various isolated parts of ponds and, excepting 0 which refers to the outlet, may be located on Map II. The last column indicates the occurrence of fish in the older ponds of the series, which are not found on the map; the numbers refer to the number of the pond in which the fish were found. ? indicates an incomplete identification.

Common Name.	Scientific Name.	Ponds.									
		1	0	5a	5b	5c	7a	7b	14a	14b	
Large-mouthed black bass	*Micropterus salmoides*	*									
Blue gill	*Lepomis pallidus.*	*									
Blue-spotted sun fish	*Lepomis cyanellus*	*									56
Pumpkin seed	*Eupomotis gibbosus*	*									
Warmouth bass	*Chænobryttus gulosus*	*									
Yellow perch	*Perca flavescens*	*				*					
Chub sucker	*Erimyzon sucetta*	*	*		*	*		*			
Spotted bullhead	*Ameiurus nebulosus*	*	*		*	*	*	*	?		
Tadpole cat	*Schilbeodes gyrinus*	*	*		*	*	*	*	?		
Pickerel	*Esox vermiculatus.*	*	*	*	*	*	*	*	*		58
Mud minnow	*Umbra limi*	*	*	*	*	*	*	*	*		58
Golden shiner .	*Abramis crysoleucas*		*	*	*	*	*	*	?		
Yellow bullhead	*Ameiurus natalis*						*				
Black bullhead .	*Ameiurus melas*						*	*	*	*	58
Dog fish	*Amia calva*										15

The perch is distributed in a still more peculiar way. It is found in Ponds 1 and 5c, but *not* in the outlet or in Ponds 5a and 5b. 5a and 5b were connected with the outlet until 1907 but 5c has probably been separated since 1851, when the L. S. & M. S. R. R. was built. I have no evidence that a sluice was ever maintained under this railroad in Pond 5. The perch is then distributed in such a way as to necessitate the conclusion, either that it was artificially introduced or that it was once in the outlet and in Pond 5a and 5b, because these make the only passage to Pond 5c where it now occurs.

The chub sucker is in all the ponds up to 7b, excepting 5a and 7a. The passage to, from, and through Pond 5a, and other points where the chub sucker occurs, was open until 1907 and the crucial collecting was done in 1908. It will be noted that the distribution of the chub sucker in Ponds 7*a* and 7*b* is entirely similar to the distribution of the perch in Ponds 5*a* and *b* and 5*c*. The chub sucker is not in the part of the long Pond 7 which has been recently connected with the outlet.

It will be noted also that the tadpole cat and the spotted bull head have not been taken in 5*a*. The yellow bullhead has been taken from 7*a* but once, but we may expect that it is a resident of the locality. One would expect to find it in Ponds 5*b* and 5*c*.

With the exception of Ponds 14*a* and 14*b*, there are no noteworthy peculiarities. The study was begun in 14*a*. This was partially drained in the winter of 1908–9 and it was therefore necessary to turn attention to 14*b*. This was probably partially drained previous to 1892 by the East Chicago ship canal, but again renewed through a dam made by the building of the Wabash railroad grade between the point of draining and the part of the pond studied about 1892. This probably accounts for the presence of only a single species in Pond 14*b*. A single juvenile blue-spotted sunfish was found in Pond 56 which is directly connected with the Calumet River where it is common.

2. *Ecological Age of Ponds.*—The ecological age of the ponds is determined by an inspection of (*a*) the amount of bare bottom, (*b*) the amount and kind of vegetation, and (*c*) the amount of humus. It is a well-established fact that an entirely new pond (in the matter of recent separation from a lake, like Lake Michigan) has little vegetation and very little or no humus. Both vegetation and humus come only with age. Age-determination is so simple that no difficulty usually is experienced by one trained in plant ecology, in arranging a series of ponds in the order of their ecological age. In the matter of the kind of vegetation we have had the advice of Dr. H. C. Cowles.

Pond 1 is the youngest, because it has the kind of vegetation that grows in young ponds, more bare sand bottom, least humus, and least vegetation. For similar reasons Ponds 5*b* and 5*c* stand second in the matter of age. Because of human interference, which has kept the vegetation down in Pond 5*c*, it is probably ecologically younger than 5*b*. The outlet is probably intermediate between 5 and 7. Ponds 7*a* and 7*b* stand next, but without any difference as far as one can see.

From the standpoint of bare bottom and humus Pond 5*a* is very much advanced. Being located near the outlet, it has become filled with decaying vegetation and a floating bog, now destroyed, indicated an advanced stage comparable with 14. It differs from 14 in possessing qualitatively "younger" vegetation and some other characters of youth. The differences between 14*a* and 14*b* have already been discussed.

In Table II. the ponds or parts of ponds are arranged in order of ecological age and the distribution of fish is shown.

TABLE II.

DISTRIBUTION OF FISH IN PONDS ARRANGED ACCORDING TO ECOLOGICAL AGE.

Common Name.	Scientific Name.	Ponds.							
		1	5	7	5*a*	14*a*	14*b*	7*b* 5*b*	5*c*
Large-mouthed black bass	*Micropterus salmoides*	*							
Blue gill	*Lepomis pallidus*	*							
Blue-spotted sunfish	*Lepomis cyanellus*	*						*	
Pumpkin seed	*Eupomotis gibbosus*	*							
War-mouth bass	*Chaenobryttus gulosus*	*							
Yellow perch	*Perca flavescens*	*	*						
Chub sucker	*Erimyzon sucetta*	*	*	*					
Spotted bullhead	*Ameiurus nebulosus*	*	*	*					
Pickerel	*Esox vermiculatus*	*	*	*	*	*		*	
Mud minnow	*Umbra limi*	*	*	*	*	*		*	
Golden shiner	*Abramis crysoleucas*		*	*	*	*			
Yellow bullhead	*Ameiurus natalis*			*					
Black bullhead	*Ameiurus melas*			*	*	*	*	*	
Dogfish	*Amia calva*								*

When the ponds are arranged according to ecological age, there is a noticeable symmetry in the distribution of the fish, and we are at once interested in its meaning.

IV. INTERPRETATION AND DISCUSSION OF THE DATA.

We have noted from the tables and discussion of tables that the present distribution of the fish is correlated with (A) the age of the ponds (Table II.) and (B) the length of time that channels have been open (Table I.).

It is a well-known fact that ponds fill with plant detritus. With the filling of ponds, the conditions change progressively in a definite direction. In a pond which remains in the same general physiographic relations during a considerable period of time, there is a succession of conditions due to the accumulation of detritus just as there is a succession of conditions in a stream due to physiographic changes.

Since the channels of communication between the different ponds have been open until recently, the present arrangement of the species of fish is very probably a *behavior adjustment* to the changed and changing conditions just so far as barriers have permitted.

We see from the arrangement and mode of origin of the ponds that our oldest pond—number 14, Map I., and Fig. 1, was once in the same relation to the lake as Pond 1 is now. At such a time it was in a condition similar to that of the present Pond 1. Ponds 5 and 7 are intermediate in conditions between Ponds 1 and 14. We have the same general basis for the discussion of ecological succession as in the streams. The changes in these ponds have depended mainly upon physiographic stability within each pond, rather than upon physiographic changes, and have been due to the *action of the organisms present on their own environment.*[1] This is true because after a given pond is once separated from the lake (Fig. 1) the changes due to the organisms go on without regard to further separation and the lowering of the lake level. Evidently the level of the water has remained much the same in the ponds after their separation from the lake proper regardless of the lowering of the lake level (Fig. 1).

The method of deducing succession herein employed is similar to that used in the case of the streams. The easily observable fact that animals occupying similar conditions are ecologically similar (*i. e.*, similar in habits and some main features of their physiology of external relations) is used as a starting point, and the conclusions drawn are to the effect that when the older habitat was in the stage of a younger habitat, it was occupied by *fishes ecologically similar to those now in the younger habitat.* Whether they were the same or different species is often of little importance to ecological succession. With this simple explanation as a background, and with the use of Figure 1, we will

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[1] The changes which are caused by the filling of the pond with plant material are physiographic, but the biological aspect is the more important, and we may discuss the changes as caused by biological forces.

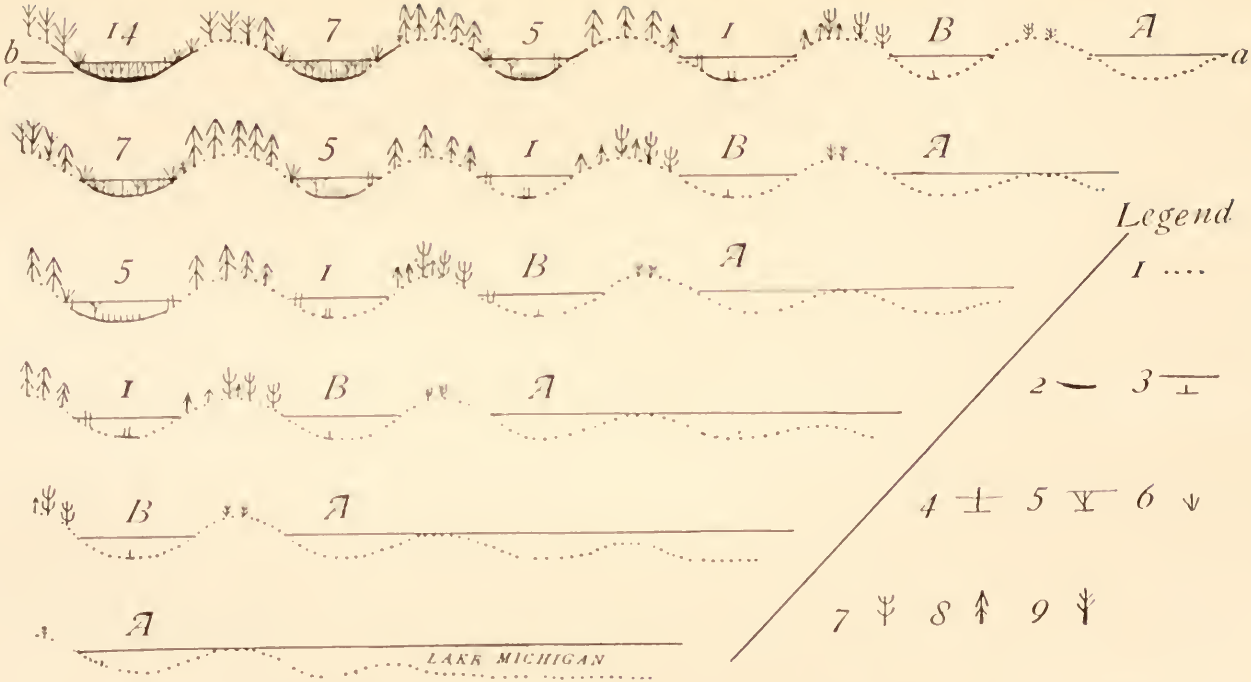

FIG. 1. Diagram showing the relation of the horizontal and vertical series of pond stages. The horizontal series at the top represents the present ponds with the intermediate ones omitted and hypothetical stages *A* and *B* added. The line *a–b* represents the ground water level; the line *a–c*, indicated by occasional dashes, the level of the lake. When read from bottom to top the entire figure represents the history of the area; showing the addition of ponds near the lake shore and the aging of the others.

The left-hand vertical series (A, B, 1, 5, 7, 14,) represents the history of the present Pond 14, and is used as the basis for the discussion of succession. The legend is as follows. (1) sandy bottom, (2) humus, (3) plants that do not reach the surface, (4) bulrushes, (5) aquatic plants which reach to the surface of the water, (6) shrubs, (7) cottonwoods, (8) pines, (9) oaks.

state, as well as practicable, the succession in these ponds with particular reference to fish.

1. *Statement of Ecological Succession.*—A statement of succession can be best made in the form of a history of Pond 14 (Fig. 1). From our knowledge of the origin of the ponds and their present topography, it is reasonably certain that there was a time when Pond 14 was more closely associated with the lake than Pond 1 is at present (Fig. 1, hypothetical stage B). At such a time Pond 14 contained less vegetation than we now find in Pond 1. For a knowledge of the ecological character of the fish which inhabit such ponds, we have collected from a pond of the same origin as those made the subject of the present study. This one has maintained a close connection with Lake Michigan and receives the waves of the lake during the spring and winter storms. It flows into the lake during every highwater period. This pond is at Beach Station, four miles north of Waukegan, Ill. Near its outer end it presents a clear bottom of sand and gravel, little vegetation and no humus. In this outer portion we have collected the pike (*Esox lucius*), which prefers clear, clean, cool water (Forbes and Richardson, '08); the red horse (*Moxostoma aureolum*), which dies quickly in the aquarium if the water is the least impure and succumbs to impure conditions in its native waters (Forbes and Richardson, '08); *Notropis cayuga*, common in Cayuga Lake and the lower course of its tributaries (Reed and Wright, '09). As compared with Illinois waters, these streams would be counted clear. We also found *Notropis cornutus*, which shows a marked preference for clear waters (Forbes and Richardson, '08).

When Pond 14 was in a very early stage (hypothetical stage B, Fig. 1) it must have been occupied by fishes which were ecologically similar to the red horse, the pike, the cayuga minnow, and the common shiner. This is a community of species which may be characterized as requiring clear, clean waters, clean bare bottom (especially during the breeding season), and little vegetation. Such fishes may be designated as *pioneer ecological types*. To this group might be added such fishes as the common perch, which is hardy and lives in a wide range of conditions.

Following the history of Pond 14 further, we note that as the vegetation grew, humus accumulated in the deeper parts, and forms ecologically similar to the black bass, the blue gill, the pumpkinseed, and the white crappie (*Pomoxis annularis*) which is widely distributed (Forbes and Richardson, '08), but which Meek and Hildebrand ('10) record only from streams and ponds which are ecologically young, must have made their appearance, if any such fishes were available. All but one of these species were taken from the pond at Beach but chiefly from the older parts. They were not clearly separated from the other species, but conditions were graded and no barriers present. We would expect the absence of some of the pioneer ecological types, such as the pike and the red horse, from such a community.

When the conditions in Pond 14 which made the habitat suitable for the last mentioned fish community had progressed a little further, *all the organisms present must have sufficiently affected the habitat to render the continued existence of some of the pioneer ecological types impossible, and at the same time prepared the way for new ecological types*, such as the blue spotted sunfish, the little pickerel, the chub sucker, the warmouth bass, the spotted bullhead and tucked away in the mud of the deeper portions a few fishes ecologically similar to mud minnows and tadpole cats.

This is the condition of our Pond 1 at present—a shallow pond with considerable accumulations of humus in the deeper parts, and much of its bottom covered with *Chara* and other plants. In this connection it should be noted that Meek and Hildebrand ('10) record the crappie and top minnow (1900) from Pond 1 (Map II.), but they are no longer found there.

It should be remembered that the parts of a pond which are so situated as to most easily allow the accumulation of plant detritus will be first to become unfavorable to pioneer fishes, while the parts which are swept clean by wave action remain in a good condition for a much longer period of time. Stages in pond development such as the present Pond 1 are the most complex of all, and are more like the larger lakes. Such stages are in a condition to support a greater diversity of ecological types than the later stages. While still retaining a part of its pioneer fishes, Pond 1 supports the forms which belong to other older conditions (mud minnow and tadpole cat).

Up to such a stage, Pond 14 must have become from the first more and more favorable to diversity of ecological types, and accordingly possessed at such a time its greatest number of species of fish. When Pond 14 was at a stage comparable to the present Pond 1, the fish community present and all the other organisms associated with it, so acted on their environment (just as they are acting on their environment in Pond 1 at present) as to make the habitat less favorable to the fish of the earlier pond stages, and more and more favorable for those dependent upon and tolerating dense vegetation, absence of bare bottom, and lower oxygen content. As a result of this action of the biota on its environment, the fishes of ecological constitution similar to that of the sunfishes and basses now present in Pond 1, disappeared either by emigration or death. In the absence of these, and in the more favorable conditions of competition and denser vegetation, fish, such as the golden shiner, were able to find a suitable habitat. At such a stage Pond 14 possessed a fish community of the ecological character of that now found in Pond 5*c*. Here the pioneer element is reduced to a single species, the perch, which is very hardy (Hankinson, '07).

The same process continued and caused the disappearance of the perch and like ecological types. A fish community ecologically like that now in Pond 5*b* (Table I., column 3) then existed. The absence of the perch in 5*b* and its presence in 5*c* may be explained, judging from the general habits of the perch (Forbes and Richardson, '08), by the fact that neither pond appears to be favorable for perch and they have been able to move out of Pond 5*b*, but not out of Pond 5*c*. An experimental comparison of the behavior of perch from Pond 5*c* and other perch habitats would have an important bearing on our problem.

The fish community of Pond 5*b* is made up of the chub sucker and the golden shiner, which are abundant, the spotted bullhead, the tadpole cat and the mud minnow. The spotted bullhead is the only one known to use bare bottom for nesting. There is only a little bare bottom in Pond 5*b*. The spotted bullhead usually builds its nest under cover (Eycleshymer, '01).

When such a fish community occupied Pond 14, the biota present gradually changed its own environmental conditions as the former stages had done. The nature of the changes are evident now when we can compare Ponds 5*c* and 5*b* with Ponds 7*a* and 7*b*. In ponds 7*a* and 7*b* we find water lilies and bladderwort in abundance. These are barely present in 5*b* and 5*c*. Ponds 7*a* and 7*b* have much less bare bottom than 5*b* and 5*c*. This is present in favorable situations very near the water's edge.

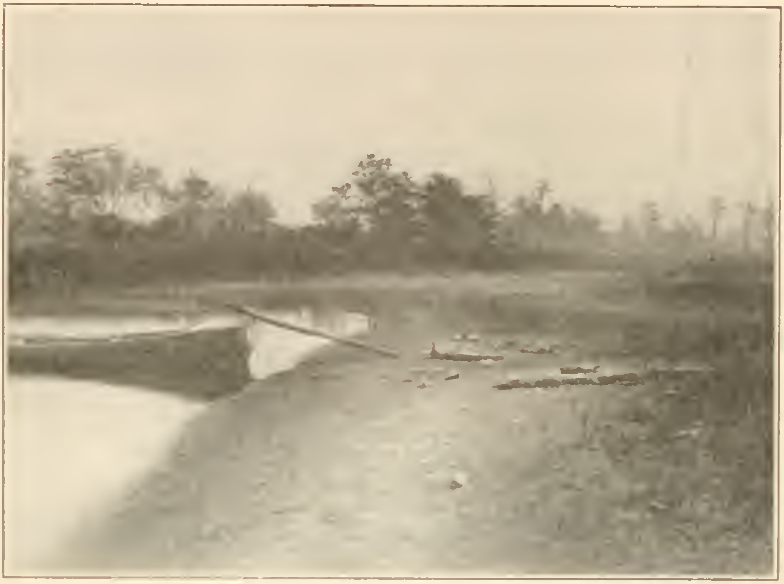

FIG. 2. Shows Pond 1 at the extreme low water of the drought of 1908. In the spring the old boat is usually covered with water. In the foreground a large area of bare sand bottom is shown, to the right a few rushes and sedges. The absence of shrubs near the water's edge should be noted.

The fish community of 7*b* is made up of the same species as the community of 5*b*, with the addition of the black bullhead. 7*a* lacks the chub sucker, and here as has been noted the chub sucker bears the same relation to the two parts of Pond 7 as the perch does to Ponds 5*b* and 5*c*. It is evident that it moved out of the pond from which a channel of exit has been open.

The first step in the transformation of the fish community of Pond 14, which was ecologically similar to the present 7*b*, into the next later stage, was the loss of such ecological types as the chub sucker and the addition of such as the black bullhead. The latter is a well-known "mud-lover" which inhabits the oldest stages of pond succession. As the conditions which organisms produced increased in intensity in the directions indicated, the species ecologically similar to the tadpole cat and the spotted bullhead disappeared, and with them probably the golden shiner also. With the loss of these we have the fish community which was in Pond 14*a* before draining. It is illustrated by Pond 5*a* also, in which the plant and animal detritus has collected because it is located at the outlet where the channel becomes shallower and narrower. Ecological types, such as the pickerel, the black bullhead, and the mud minnow make up this community in Pond 14. They usually continue until the pond becomes temporary when they are destroyed by drying.

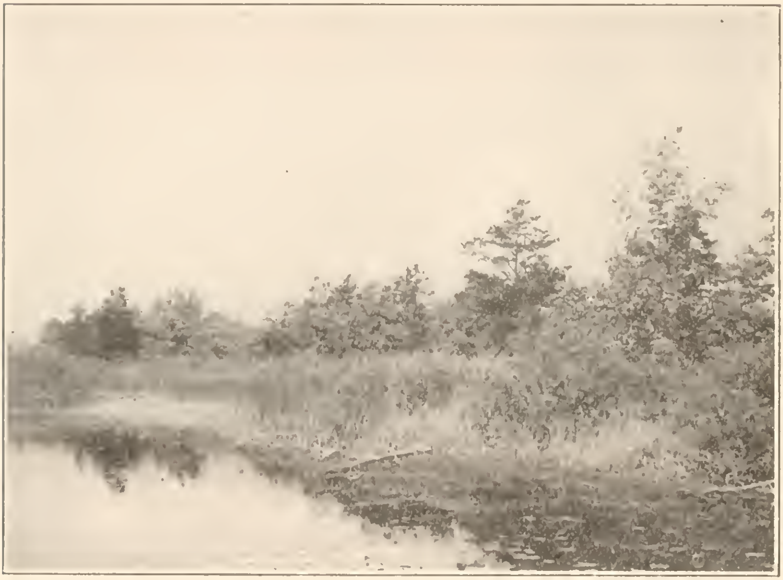

FIG. 3. Showing Pond 14 at moderate low water. In contrast with 1, we see that it is choked with vegetation and the margin occupied by shrubs and bulrushes, etc.

2. *The Future of the Pond.*—Ecology is one of the few biological sciences in which prediction is possible. I shall venture to predict the fate, in a state of nature, of a pond like Pond 14*a* *was* at the

point where the study was first begun, before the draining took place.

Had Pond 14*a* not been disturbed by man, it would have continued to fill with humus. Its center would have come to be occupied by the cat tails, bulrushes, *Proserpinaca*, and *Equisetum*, all of which invade from the sides. When such a stage was reached it would be subject to almost complete drying in extreme droughts and the fish would be eliminated in the order of their ability to withstand draught, and in relation to accidents of distribution in parts of the pond drying to various degrees.

In September, 1908, we were able to obtain evidence on this point from some of the older ponds. A group of black bullheads, grass pickerel and mud minnows were found in the lowest part of one of the sections of pond 58. The pickerel were dead and badly decayed. The bullheads were dead and just beginning to decay, but the mudminnows, fully a bushel of them, were still alive although without water. This lot included some of the largest individuals collected.

When Pond 14 reached such a stage, its fish content would become very problematical, dependent upon the distribution of the different species with respect to deeper and shallower parts of the pond, and the length of the drought, as related to the resistance of the different species. Evidence for this is seen in Pond 14*b*, where *Ameiurus melas* is the only species present since a probable partial drying of the pond.

Indeed, we may carry the prediction one step farther. When such a pond becomes of this sort the plants that take hold of it are the grasses, sedges, etc., which fill the soil with roots, and form hummocks. If accessible to them, such ponds become the breeding place of the dog fish. During the drought of 1908 I found a school of half grown dog fish in such a pond, which answered the description of the favorite breeding place of the dog fish as given by Reighard ('02). Such a stage as this will be followed by the invasion of shrubs and the final destruction of the pond as an aquatic habitat.

We have in ponds a progressive change in the conditions and a progressive change in the ecological and physiological character of the fish communities, and a succession or evolution of fish communities through emigration, death, immigration, and the modification of *mores* by external stimuli.

V. ECOLOGICAL SUCCESSION AND SUCCESSION OF SPECIES.

The difference between ecological and geological succession were suggested in my preceding paper. We noted that ecological succession deals with the succession of *ecological types*. We noted also that geological succession is due, in the main, to the death of a given set of *species* and the evolution of new ones throughout geological time. While this is true of the broader aspects, in the more detailed cases and especially in dealing with recent post-glacial fossils, the palæontologist often encounters a vertical succession of fossils which have been left behind by the migrations of a succession of species over the locality of fossilization (Warming, '09, p. 362, Adams, '05 and '09, Sharff, 07, esp. Chap. IX. and citations). Palæontologists may also encounter vertical succession of fossils in situations where such succession as we have been describing has taken place.

1. *Vertical Succession of Fossils.*—Steenstrup ('41) found from the study of fossils in moors that one kind of vegetation succeeded another. Various other workers (Andersson, '97) have found similar arrangements. In the case before us the fossilization of species would give a vertical succession of fossils.

Turning again to the diagram, we note that the skeletal parts of any fish in the earliest stages indicated by hypothetical stage B might have been preserved as fossils. The accumulations of humus which lead up to stage 1 would have covered the fossils of stage B, the fish of stage B would be present, if at all, as fossils at the bottom of the pond.

Likewise, the accumulations of humus which led to stage 5 covered those skeletal parts of the fish of stage 1, and the fish of stage 1 should be found as fossils overlying those of stage B and underlying those of stage 5. Again, the accumulations of humus which led up to stage 7 covered fossils which were present and added at stage 5, and the fish preserved as fossils from stage 5 would lie above all those preserved as fossils from the younger stages and below those of stage 7. Fossils from stage 14 and the intermediate stages would lie at the top of the series and above those of stage 7.

However, no such arrangement of fossils has been found in these ponds. No attempt to ascertain whether or not they are present has been made.

2. *Relation to Climatic Changes.*—As has been indicated, succession is due to changes in conditions which make it impossible for a given group of organisms to continue to live at a certain locality. Accordingly, such a group gradually disappears and is gradually succeeded by a group which is adapted to the new conditions.

The changes in conditions referred to are climatic or physiographic changes, and others independent of physiography and climate. The last type of change is usually due to the action of the organisms themselves. These three forces may act separately or together. The latter is probably the more common. In case the changes are climatic, both the ecological succession and the succession of species over a locality might be partly due to migration of a succession of species. Narthorst ('70, fide Warming, '09, p. 362) found that the oldest fossils underlying moors are of arctic tundra plants. In post-glacial dispersal arctic-tundra plants and animals were succeeded by conifers and associated animals, and by deciduous trees and associated animals (Adams, '05).

3. *Relation of Horizontal Series and Vertical Succession.*—Cowles ('01) pointed out the fact that there is frequently a horizontal series of successional stages (habitats of different ages) associated with continuous physiographic processes, such as the deposition of sand along shores. The diagram (Fig. 1) illustrates both the horizontal and vertical series of conditions with which we have been dealing. Cowles ('01) further pointed out that the horizontal series of plant communities must bear a close ecological resemblance or be practically identical with the vertical series of past plant communities which have succeeded one another over a given locality in the older part of the horizontal series. He pointed out further that the horizontal series may be taken as an index of what the vertical series has been. He would except the first stages of plant communities which occupied a locality at the close of the ice age and which as has been stated, are arctic plants. These arctic plants, however, must have affected

the soil or pond bottom in much the same way as the pioneer plants of the present climate would affect them. The differences resulting from the change of climate are then, those of detail rather than of principle.

4. *The Relation of Ecological Succession to Species of Fish.*—If fish were found fossil in the bottom of Pond 14, in the order which we have indicated, one might conclude at once that it constituted a proof of ecological succession. This seems to have been the general impression of zoölogists who have heard the presentation of these data. The question has been asked, "Do you find fossil in 14 all the fish which you mention as occurring in succession there?" My answer to the effect that no such fact has been discovered seems to have been regarded as constituting a refutation of the entire statement of ecological succession.

If fish were found fossil in the order described above, and the species and order of species, the same as now found in the horizontal series of ponds, we would have some important data bearing on succession, migration, and other matters of interest to be discussed presently, but this would yield *no crucial evidence for or against ecological succession*. Ecological succession is based upon physiology, habits, behavior, mode of life, and the like, which I have proposed to call *mores* (opposed to the term form). Unless the mores of the morphological species found fossil were the same as the *mores* of the same morphological species at present, they would have no weight in the matter, and it would be impossible to ascertain mores from fossils if such fossils were found.

If the same one or more species were found fossil in each and all of the vertical stages of a pond like 14, the evidence would not refute the proposition of ecological succession because the physiological characters of the individuals of a given species living in the early stages could have been very different from those of individuals living in later stages, without the differences being shown in the preservable skeletal structure. Furthermore the modifiability of animal behavior seems well established. The same species of plant may remain in a number of different pond stages. Such plants show suitable functional responses manifested by different growth-forms in different stages.

The proof of ecological succession must rest on the results of experimental work on the animals of the different stages of such a horizontal series. *It matters not from the point of view of ecological succession what the forms are, so long as the mores are different in the different stages of the horizontal series, and the character of the mores is correlated with the environmental conditions.* When such differences and relations are found, ecological succession is essentially established. It is of course recognized that within rather uncertain limits the *mores* of a morphological species remain, in a general way, the same throughout its geographic range. This kind of reasoning must be pursued with caution. When applied in detail or to "common" species which are wide ranging, it is not at all trustworthy. If, for example, an examination of the deposits in the bottom of a pond, such as Pond 14, showed the presence of a fish which now habitually inhabits the *youngest stages* of pond succession, let it be a more northern, or the same species as those in the horizontal series under consideration, it would constitute some evidence for ecological succession which could be further checked by the study of the modifiability of the *mores* of that species at present. However, in no case can this kind of evidence be crucial.

Furthermore, the conditions such as were in primaeval Pond 14 could have been reached by a pond like Pond 1 in a few hundreds of years. In a pond with a soil more suitable than sand for the growth of aquatic plants, these changes could take place within the life time of the builder of such a pond (Knauthe, '07, p. 575).

VI. GENERAL DISCUSSION.

We noted in the preceding paper that physiographic analysis is a method. Here we have employed another method which, while not physiographic, is similar in principle, since it deals with succession over a given locality. The cause of this succession is biological and the biological interest is proportionately greater.

We have here the same attempt to discover something of the physiological character of the organism as a whole, and to classify organisms on the basis of the conditions in which they are most nearly in physiological equilibrium. Again, the *mores* of the fish of pond stages in other parts of the world are probably similar if the ponds are similar.

However, a legitimate question at this point would be: "What is the value of all this reconstruction and these complicated conceptions to biological science and the analysis of the organism?" Indeed, this is a question which we have asked ourselves repeatedly while working through the necessary plant data and all the scattered unorganized literature with which the investigator in this line must deal. The question has always been answered in a manner satisfactory to us because whatever value the papers on the ponds of the south end of Lake Michigan and other similar papers may possess, is due to the conception of pond succession acquired from the plant ecologists. Had I not acquired this knowledge I would have done as others have done—worked this excellent area over without seeing anything in it. The problems that have arisen in connection with this work have given a fresh point of view and a motive for investigation which has repaid the effort.

In connection with problems and motive for their solution, it should be noted that this is one of the most important things which the plant ecologists regard as of value in the conception of succession. Cowles, who has done more to stimulate work on succession and the use of physiographic method than any other American ecologist, regards functional response of plants (determined by watching plants and experimenting on plants) as much more fundamental than succession. This appears to be generally true of plant ecologists. Warming, who has been a great leader in ecology, emphasizes the physiological side.

We have, then, among the workers and originators of the use of this principle, a condition with respect to its place in biology which is quite different from what one would expect from simply noting what has been the dominant thing in their work. The relation of the historical and genetic side of ecology to more fundamental physiological and ecological problems, is similar to that of the historical and phylogenetic side of evolution, to the problems of biology and the motives for investigation. When Darwin framed the idea of evolution into a logic which was not refutable by academic attack, the facts of biology took shape, arranged themselves into orderly relations to each other. This made possible methods of work which were new, opened up new problems, the attempt at the solution of which has made modern biology what it is.

If we assume the attitude that nothing can be done in the organization of natural history materials into a science, we are closing the question to investigation, much as the idea of special creation closed other lines of biological investigation. Still an occasional biologist appears to take this point of view.

Aside from the general questions which we have been discussing, there are many practical applications of pond successsion to the study of behavior and to the economic and quantitative side of biology. These we will discusss in a succeeding paper under the head of the causes of succession in ponds.

VII. SUMMARY.

1. There is a series of ponds at the south end of Lake Michigan arranged in the order of their age; age is determined by the physiographic history; by the relative amount of humus and bare bottom, and by the quality and quantity of vegetation.

2. Species of fish are arranged in these ponds in an orderly fashion; the order is related to the age of the ponds.

3. The ponds of different ages represent stages in the history of older ponds.

4. The horizontal series of fish communities is ecologically representative of the succession of fish communities within the older ponds.

5. The method employed here is similar to physiographic analysis; the motives and possible results are similar but more strictly biological, because the causes of the succession are the organisms themselves.

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UNIVERSITY OF CHICAGO,
April 19, 1911.

VIII. ACKNOWLEDGMENTS AND BIBLIOGRAPHY.

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Hildebrand; some of the collecting was done in coöperation with Dr. Meek and Mr. Hildebrand. Without the assistance of these gentlemen this paper could not have been written. The writer is also indebted to Mr. Ellis L. Michael for the reading of the manuscript.

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A HETEROCHROMOSOME OF MALE ORIGIN IN ECHINOIDS.

DAVID HILT TENNENT.

The observations of Baltzer ('09) seem to have led to an immediate conclusion that in echinoids the female is the heterogametic sex while the male is homogametic. My observations on the $\frac{\textit{Hipponoë}\ ♂}{\textit{Toxopneustes}\ ♀}$ cross, taken in conjunction with those of Heffner ('10) and Pinney ('11) show that this conclusion cannot be final. The study of the material mentioned has given convincing evidence that in *Hipponoë esculenta* (*Tripneustes esculentus*), a heterochromosome is carried by half of the spermatozoa and that the dimorphism of somatic chromosome groups in in straight fertilized *Hipponoë* and $\frac{\textit{Hipponoë}\ ♂}{\textit{Toxopneustes}\ ♀}$ material must be correlated with dimorphism of the spermatozoa.

The heterochromosome in question is hook-shaped in form (*H* Fig. 1, *A*, *H* Fig. 2, *B*). In a study of fifty-two spindles in which I was able to reach a definite conclusion as to the presence or absence of the hook-shaped element, it was found to be present in twenty-eight and absent in twenty-four instances (Fig. 3).

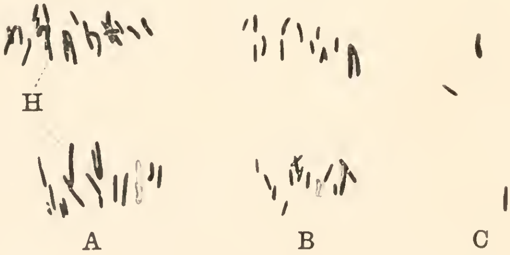

Fig. 1. *Toxopneustes* ♀ × *Hipponoë* ♂. Three longitudinal sections of one spindle in anaphase. In *A* and *B* two chromosomes left in outline for sake of clearness. 33 chromosomes shown. Hooks in side view. ×1,500.

Miss Pinney ('11) has been able to show the occurrence of this element in half of the straight fertilized *Hipponoë* eggs. The non-occurrence of such an element in *Toxopneustes* eggs has been shown by Heffner ('10) and by further study in my laboratory with this point in mind. I have been able to determine that this hook-shaped chromosome is present in half of the *Toxopneustes* eggs which have been fertilized by *Hipponoë* sperm.

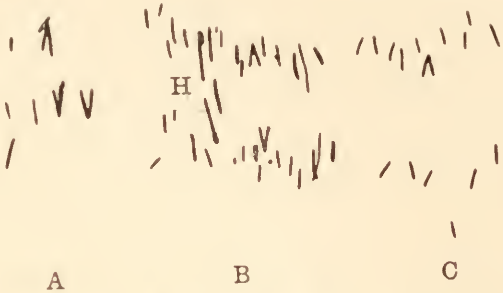

FIG. 2. *Toxopneustes* ♀ × *Hipponoe* ♂. Three longitudinal sections of one spindle in anaphase. 31 chromosomes shown. Hooks in front view. ×1,500.

By this analysis of the subject it is therefore shown: (1) That the hook-shaped chromosome is peculiar to *Hipponoe*, (2) that this chromosome is carried by the *Hipponoe* spermatozoa, and (3), that, since this element is found in half of the straight fertilized eggs and in half of the cross fertilized eggs it must be present in but half of the spermatozoa.

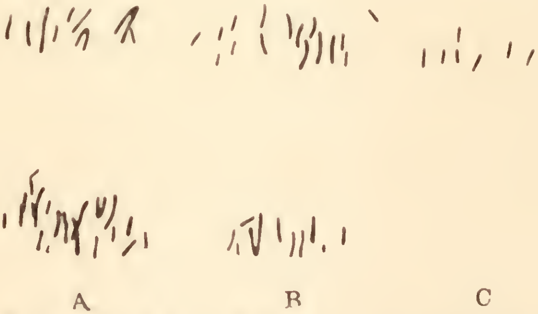

FIG. 3. *Toxopneustes* ♀ × *Hipponoe* ♂. Three longitudinal sections of one spindle in anaphase. 32 chromosomes shown. No hooks present. ×1,500.

No conclusive statement can as yet be made regarding the number of chromosomes in these eggs. The old idea that the somatic number of chromosomes in echinoderms is either eighteen or thirty-six is evidently incorrect. Pinney ('11) has shown for *Hipponoë* 32 or 33. Heffner ('10) counted 36 for *Toxopneustes*. Pinney ('11) counted 37 or 38 in anaphase plates in *Toxopneustes*, counts which I was able to verify. In the cross fertilized eggs figured in this paper the counts vary. Fig. 1 shows 33; Fig. 2 shows 31; Fig. 3 shows 32.

Miss Pinney ('11) has given as full a discussion of the subject as the facts known at this time warrant. For a conclusive resumé a full knowledge of the development of the germ cells in these forms now seems almost imperative.

BRYN MAWR COLLEGE.

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HETEROCHROMOSOMES IN THE GUINEA-PIG.

N. M. STEVENS.

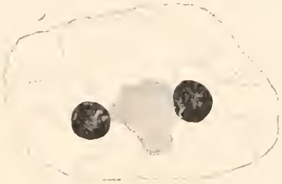
In view of the fact that the guinea-pig is so much used for experimental breeding tests in Mendelian heredity, it seemed desirable to secure some further knowledge of the behavior of the chromatin in the germ cells. An attempt has therefore been made to follow through the spermatogenesis, and a brief note on the subject was published in the BIOLOGICAL BULLETIN, Vol. XX., No. 1, January, 1911. The material has proved to be very unfavorable, and the results are not so satisfactory as are to be desired. As Meves ('99) has given a very full account of the transformation of the spermatids into spermatozoa, I shall omit all discussion of that phase of the spermatogenesis, and confine my attention to the behavior of the chromatin in the nuclei and mitoses of spermatogonia, spermatocytes and spermatids.

METHODS.

At first the testes were fixed by several methods which had given good preparations of other spermatogenesis material. It soon became evident that only the Flemming and Hermann osmic mixtures gave even fairly good fixation of the chromosomes in mitosis, and also that the testes must be removed from the animal, cut into small pieces, and transferred to the fixing fluid with great rapidity in order to secure good results. Meves' method of using the fixing solutions at 35° C. proved to be an improvement on the use of cold solutions. The sections, 5μ thick, were stained with either thionin or iron-hæmatoxylin, orange G. being used in some cases as a plasma stain. Thionin, or thionin and orange gave the most satisfactory results, especially for the chromosomes in mitosis. Aceto-carmine preparations were made for each set of fresh material and proved valuable. It was found that pieces of the testis macerated with needles in aceto-carmine would keep in fairly good condition in a tightly stoppered vial for several weeks.

SPERMATOGONIA.

As in other vertebrates the testes consist of much coiled tubules in which, in mature animals, spermatogonia and Sertoli-cells are found at the periphery against the wall of the tubule; then come first and second spermatocytes in various stages, spermatids, and unripe spermatozoa attached to long processes extending from the Sertoli-cells toward the center of the tubule, ripe spermatozoa being found in or near the lumen of the tubule.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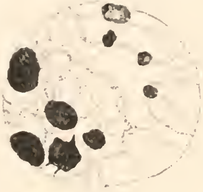
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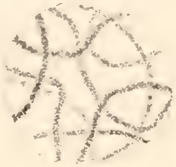
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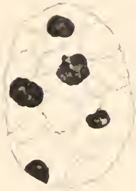
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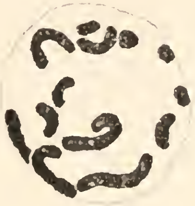
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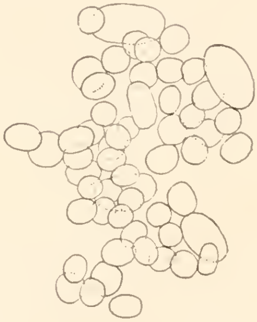
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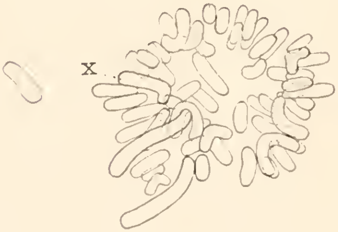

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FIG. 1. Nucleus of Sertoli cell.

FIG. 2. Nucleus of spermatogonium.

FIG. 3. Spireme stage, spermatogonium.

FIGS. 4 and 5. Nuclei from a young testis, rest-stage and prophase.

FIG. 6. Fifty-six chromosomes of a spermatogonial prophase. Aceto-carm. prep.

FIG. 7. Equatorial plate of a spermatogonial mitosis; 56 chromosomes. All figures 2,000 × 1½, reduced ½.

In one very young animal only spermatogonia were found, Sertoli-cells not having been differentiated, and no maturation stages having yet appeared.

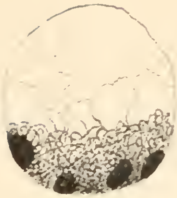
The nuclei of the Sertoli-cells (Fig. 1) are clearly differentiated from those of the spermatogonia. They are larger and usually flattened parallel with the wall of the tubule. In the center of the nucleus is a large plasmosome and closely associated with it one, two or more chromatin nucleoli, or karyosomes, which often appear to have a central vacuole. The reticulum of the nucleus does not stain with thionin or iron-haematoxylin.

The larger number of the spermatogonial nuclei contain large clumps of chromatin of various sizes, and tangled threads into which the clumps are gradually resolved after a mitosis (Fig. 2). Shortly before a mitosis all of the clumps disappear and a perfect spireme stage is formed (Fig. 3). In a young testis nearly all of the nuclei are similar to Fig. 4. A few are in spireme or prophase stages (Fig. 5). It has proved a very difficult matter to determine the number of chromosomes either in the spermatogonia or spermatocytes. I have never found a case of a perfectly flat plate where there was no overlapping. After spending a great deal of time over this one point, I have concluded that 56 (Figs. 6 and 7) is probably the correct number. Fig. 6 shows in outline the chromosomes of a prophase, from an aceto-carmine preparation, the cell having been much flattened. Two large pairs can be distinguished, and the others vary considerably in form and size. Fig. 7 is the best equatorial plate which was seen either in aceto-carmine preparations or in sections. This was taken from Hermann material stained with thionin. It will at once be seen that the chromosomes overlap in such a manner as to make accurate counting difficult. The one aberrant chromosome is not a characteristic feature. One unusually long pair of rods is conspicuous. There are several V's, and the remainder have the form of straight or somewhat curved rods. The two long rods are always noticeable in an anaphase, lagging behind the shorter chromosomes. The V's are probably the same chromosomes which have the spindle-fibers attached centrally and pass to the poles as V's in the first maturation mitosis. The heterochromosomes one cannot distinguish at this stage with certainty; the smaller one is one of the smallest chromosomes, perhaps *y*, and *x* which seems to have no mate may be the larger.

First Spermatocytes.

The nuclei of the youngest spermatocytes are similar to those of the spermatogonia (Fig. 2), clumps of chromatin and fine threads. Before the clumps have disappeared the chromatin threads are more or less massed at one side of the nucleus (Fig. 8) in a synizesis stage, which resolves itself into an irregular bouquet stage (Fig. 9) with loops much coarser than the threads of the previous stage. Frequently one dense chromatin mass is seen half hidden among the bases of the loops. Some of the loops look as though formed by telosynapsis, but if this is so, the halves of the bivalent loops must later become parallel, for the bouquet stage passes over into a parasynaptic stage (Fig. 10 and Fig. 11) which is followed by a spireme stage (Fig. 12) in which there is no longer any evidence of synapsis. In the spireme stage there are always small masses of dense chromatin-like material lying against the nuclear membrane, and staining as deeply with thionin as the chromosomes in mitosis. A large heterochromosome *x* is always present, and one or more plasmosomes can usually be found in sections. In early prophases (Figs. 13 and 14) the chromatin appears as though gathering together about definite centers along the spireme, leaving linin threads between. Sometimes a longitudinal split is visible in some of the larger chromsomes (Fig. 14.) In several cases at this stage the two heterochromosomes (*xy*) have been clearly separated as in Figs. 13 and 14. In Fig. 13 a pale plasmosome is also present.

In most first spermatocyte spindles two chromosomes are conspicuous (Fig. 15). One of these (*xy*) is the unequal heterochromosome, usually bilobed at the larger end (*x*). The other (*a*) is larger than any of the others and without doubt is composed of the two long rods of the spermatogonia. As a means of determining the reduced number of chromosomes, a number of spindles in the best material were selected, and an attempt was made to draw all of the chromosomes which were divided between two sections. Twenty-eight were found in a number of the clearest cases. These are all shown for the spindle of



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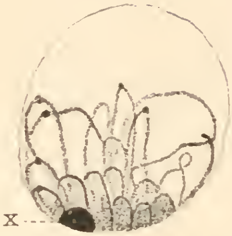

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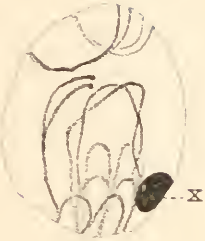

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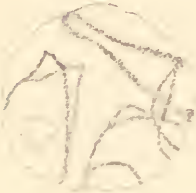

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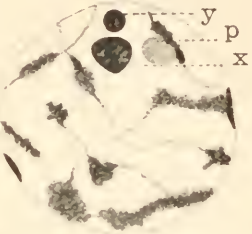

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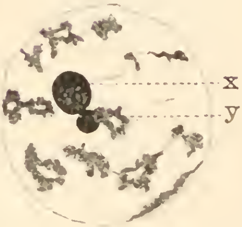

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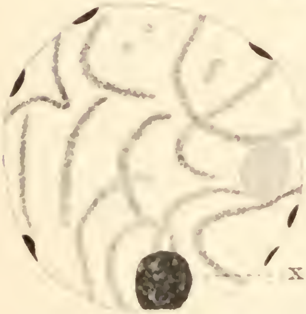

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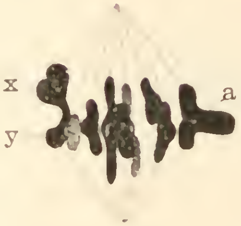

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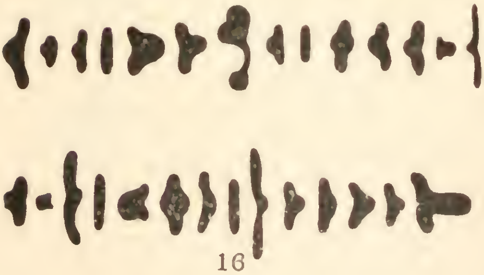

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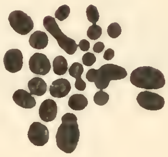

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FIG. 8. Synizesis stage.

FIG. 9. Bouquet stage; x, the heterochromosome.

FIGS. 10 and 11. Later stages showing parasynapsis.

FIG. 12. Spireme stage.

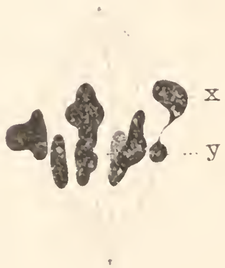
FIGS. 13–14. Prophases showing the unequal pair of heterochromosomes (*x*, *y*).

FIG. 15. First spermatocyte metaphase; *x*, *y*, the heterochromosome pair; *a*, the largest chromosome.

FIG. 16. The 28 chromosomes from the spindle of Fig. 15.

FIG. 17. First spermatocyte equatorial plate, 28 chromosomes.

which Fig. 15 is a part, in Fig. 16. Many of them are evidently parasynaptic pairs attached to spindle fibers at one end, others are similar pairs attached in the middle and pulling apart in the



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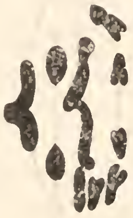

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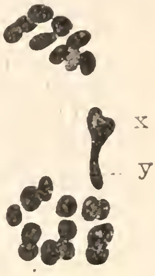

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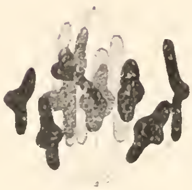

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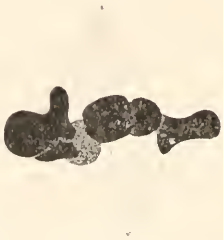

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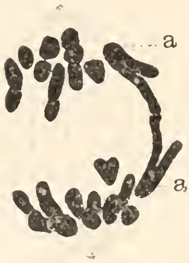

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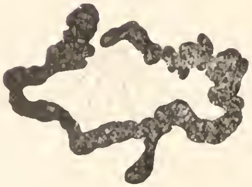

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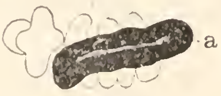

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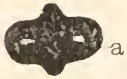

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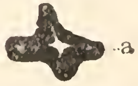

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FIG. 18. The heterochromosome pair (*x y*) dividing.

FIG. 19. Metakinesis.

FIG. 20. Anaphase. Heterochromosome pair still undivided.

FIG. 21. Newly formed spindle; chromosomes well advanced in metakinesis.

FIG. 22. Older spindle; metakinesis hardly begun.

FIG. 23. Spindle forming with chromatin in form of a thick spireme.

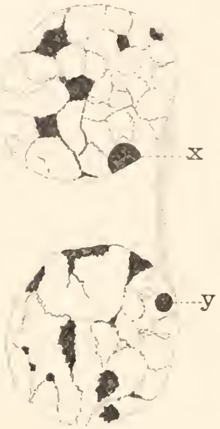
FIG. 24. Anaphase; chromosome *a* separating as a pair of V's.

FIGS. 25–27. Chromosome *a* in the form of a split band, passing into a cross-form in metakinesis.

form of crosses. The rods are probably lateral views of cross-shaped forms. Fig. 17 shows 28 chromosomes in an equatorial plate. Fig. 18 is another metaphase in which the members of the heterochromosome pair are already separating earlier than the others. In Fig. 19 the separation of some of the chromosomes as rods and V's in metakinesis may be seen. Fig. 20 is a late anaphase in which the heterochromosome pair is still undivided. This is unusual, but shows a kind of variation which is noticeable through all the stages of the spermatogenesis. Variation is most conspicuous in the prophases of the first maturation mitosis. As a rule one finds such early prophases as are shown in Figs. 13 and 14, and the chromosomes of the young spindle (Fig. 21) are well on their way in the process of metakinesis, but one sometimes finds the chromosomes of an older spindle (Fig. 22) much less far along toward division than in Fig. 21, and not infrequently I have seen a condensed spireme, as in Fig. 23, free in the cell, the nuclear membrane having disappeared, and a spindle forming. These variations must, I think, be an expression of the difference in rate at which maturation takes place at different times, and probably under different stimuli, or stimuli of different intensity. Fig. 24 is an interesting anaphase showing the large chromosome pair separating as a pair of V's instead of a pair of rods, as would have been expected from Fig. 15*a*. In the preparations from one animal I repeatedly saw the largest chromosome as shown in Figs. 25, 26 and 27, a split band forming a cross in a later stage (Fig. 27). In all other material the largest chromosome has appeared as in Fig. 15, which, together with Fig. 24, suggests that the four ends of the two long chromosomes which form the pair are usually folded together. The two lateral halves of the cross shown in Fig. 27 folded together would give the form shown in Fig. 15*a*, and might then separate as in Fig. 24. The same kind of folding has very likely occurred in chromosomes 5 and 19 in the series shown in Fig. 16, and possibly in other cases where it is less evident.

SECOND SPERMATOCYTES.

Between the two maturation mitoses there is a rest stage in which the pairs of second spermatocytes may often be found connected by spindle fibers as in Fig. 28. This figure shows the larger (x) and smaller (y) heterochromosome with smooth outline, and the remainder of the chromatin in the form of irregular clumps and strands. In some cases second spermatocyte spindles have been found associated with first spermatocytes in mitosis in such a way as to indicate that the rest stage between the two divisions may often be omitted. This is as far as the heterochromosomes can be traced.



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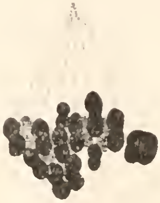

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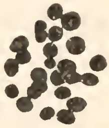

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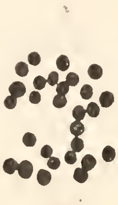

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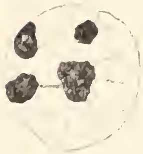

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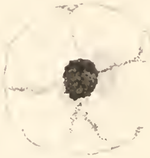

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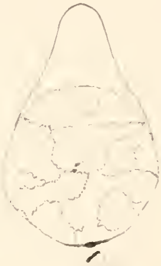

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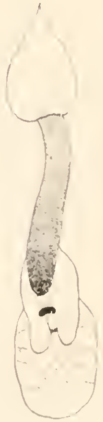

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FIG. 28. A pair of second spermatocytes showing the heterochromosomes x and y.

FIG. 29. Second spermatocyte metaphase.

FIG. 30. Second spermatocyte equatorial plate.

FIG. 31. Second spermatocyte anaphase.

FIGS. 32–35. Four stages in the transformation of the spermatid, showing no trace of the heterochromosomes. All figures 2,000 × 1½, reduced ½.

In the second maturation spindle I have never been able to count the full number of chromosomes because of crowding and overlapping. In the clearest cases seen in aceto-carmine preparations, the chromosomes are dumb-bell shaped, as in Fig. 29. They are, however, rarely so well separated. Fig. 30 shows 24 out of the 28 which should appear in such a plate, and Fig. 31 an anaphase in which no attempt was made to draw all of the chromosomes. These figures, though valueless as a demonstration of the number of chromosomes or of the division and distribution of the heterochromosomes, are given to show that in the guinea-pig there is no such second synapsis or numerical reduction as has been described by Guyer ('09, '10) for the guinea-chicken, the domestic chicken and for man, also by Jordan ('11) for the opossum. There are plenty of figures in the sections which show that the number is certainly not reduced one half, and probably not reduced at all.

The Spermatids.

In the youngest spermatids as in other stages immediately following mitosis, the chromatin appears in irregular clumps (Fig. 32). The most characteristic spermatid stage is shown in Fig. 33; here there is a large plasmosome, and closely associated with it in the center of the cell a large mass of chromatin from which strands of varying thickness radiate to the nuclear membrane. In a somewhat later stage (Fig. 34) the central clump of chromatin has all disappeared, and the nucleus contains only a faintly staining reticulum. In the half grown spermatozoa, attached to the Sertoli cells, the nucleus is finely granular (Fig. 35). This figure was drawn from a preparation stained with thionin. The ring was stained a deep blue, and the nuclear granules also blue, deeper at the proximal end. These figures are given as evidence that the spermatids and spermatozoa are not visibly dimorphic, each of the figures being typical for all of the nuclei of the stage which it represents.

Summary.

1. The probable number of chromosomes in the spermatogonia of the guinea-pig is 56, and in the spermatocytes 28.

2. Synapsis is of the parasynaptic type.

3. A typical heterochromosome is present in the growth stages of the first spermatocytes. In metakinesis this is separated into a larger and a smaller component, as in many insects.

4. The second spermatocytes are visibly dimorphic in the rest stage, one containing a larger, the other a smaller heterochromosome.

5. The spermatids and spermatozoa are not visibly dimorphic.

Discussion.

One or more unpaired heterochromosomes have been reported by Guyer ('09, '10) for the guinea-chicken, domestic chicken and man, and by Jordan ('11) for the opossum. This is the first case, so far as I am aware, in which an unequal pair of heterochromosomes has been found in a vertebrate. Although it has not been possible to follow the heterochromosomes through the second maturation mitosis, there is no evidence against the supposition that every chromosome divides equally in that mitosis, giving spermatids one half of which contain a division product of the larger heterochromosome, the other half one from the smaller heterochromosome. If it can be shown that the female guinea-pig has 56 chromosomes, two of which correspond to the larger heterochromosome of the male, then the fertilization formula will be like that for similar cases in insects.

	Eggs.		Spermatozoa.		Zygote.
Gametes	$27 + X$	+	$27 + X$	=	$54 + XX$ = ♀
	$27 + X$	+	$27 + Y$	=	$54 + XY$ = ♂

This is what is to be expected, but, in view of recent developments in echinoderm spermatogenesis, it is at any time possible that we may find other conditions; for example,—

	Eggs.		Spermatozoa.		Zygotes.
Gametes	$27 + Y$	+	$27 + X$	=	$54 + XY$ = ♂
	$27 + Y$	+	$27 + Y$	=	$54 + YY$ = ♀

In this case the female would still be homozygous but the male would have the excess of chromatin, realizing in that respect McClung's ('02) original suggestion in regard to the "accessory" in the Orthoptera; and the spermatozoön containing the X chromosome would be male-producing instead of female-producing.

It has always seemed to me somewhat improbable that the small heterochromosome of an unequal pair owes its smaller size to gradual degeneration, and much more likely that the unequal pair may, at least in some cases, be composed of an equal pair of chromosomes with an unpaired heterochromosome fused with one member of the pair, as McClung ('05) has shown to be the case in Hesperotettix.

The definite cases of regulation in the number of heterochromosomes in the male-producing eggs of *Aphis* and *Phylloxera;* and the case of *Rhabditis nigrovenosa* (Boveri, '11; Schleip, '11) where a hermaphrodite with 12 chromosomes produces spermatozoa with 5 and 6 respectively, by rejecting one heterochromosome in the second maturation division, naturally suggest that the origin of the unpaired heterochromosome in the male may be attributed to a similar regulatory process occurring somewhere in the evolution of a hermaphrodite organism, and giving male and female-producing spermatozoa. Such a regulation might give at first males and hermaphrodites, a not uncommon condition, but degeneration of the male reproductive organs in the hermaphrodite would result in the usual bisexual condition with the sexes equal in numbers, unless the sex ratio were changed by an environment discriminating against one sex, or by some regulation in the number of male and female-producing spermatozoa. The unequally paired condition of the heterochromosomes might easily have come about by fusion of the heterochromosomes in both sexes with another pair of chromosomes in the course of a general reduction in the number of chromosomes characteristic of the species. It is much more difficult to see how the changes necessary to bring about the condition indicated in the second formula could occur, since we must account either for the disappearance of three X chromosomes leaving only the one X in the male, or for the appearance of an X chromosome *de novo*. Payne's ('09, '10) figures for several of the Reduviidae suggest other complications.

Some recent observations on other material have impressed upon me the probability that the chromatin units representing sex and other characters may be very small indeed, and that in such cases as that of *Culex* (Stevens, '11), where no heterochromosome differentiation of any kind has been detected, the members of a pair of chromosomes may differ by a sex unit or some other character unit, and the difference in size come within the probable errors of most careful observation.

Were it not for a few cases of condensation of an apparently equal pair of heterochromosomes in the male germ cells (Lepidoptera, Stevens, '06, Dederer, '07, Cook, '10; *Anisolaba maritima*, Randolph, '08), and in the female germ cells (*Aphrophora*, Stevens, '06, *Rhabditis nigrovenosa*, Boveri, '11), one might suspect that the condensed condition of the odd chromosome and the unequally paired heterochromosome of the growth stage of the first spermatocytes might be due to their unpaired or unequally paired condition preventing them from joining with the other bivalent chromosomes to form a spireme, especially in cases of parasynapsis. In *Culex* no such difference is effective in bringing about a condensed condition of one pair of chromosomes and in *Anopheles*, where the difference in size of the heterochromosomes is slight, condensation is much less complete than in most cases. In the guinea-pig condensation is complete from the synapsis stage on.

In the guinea-pig the condensation of the heterochromosome pair and its behavior in mitosis are as typical as in *Tenebrio* and many other coleoptera previously described by the author, and only the large number of chromosomes of such a consistency that good fixation in mitosis is difficult to secure, makes it impossible to give as complete a demonstration of the relation of the heterochromosomes to sex as in many other forms.

Bryn Mawr College,
May 23, 1911.

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A STUDY OF THE CHROMOSOMES OF HIPPONOË ESCULENTA AND MOIRA ATROPOS.

EDITH PINNEY.

The comparatively recent investigations made on fertilized echinoderm eggs with especial regard to the question of chromosome individuality had their beginning in an attempt to correlate the behavior of the chromosomes during fertilization and the subsequent cleavage stages with the observed facts of parental dominance in hybrid cultures.

The first observations are recorded by Tennent, '07. He figures the equatorial plates of *Toxopneustes variegatus*, *Moira atropos* and *Arbacia punctulata* in which individual characteristics for each species are evident. The contrast between the chromosome groups in *Moira* and *Arbacia* is so apparent that in the equatorial plates of the hybrid $\frac{\textit{Arbacia}\ ♂}{\textit{Moira}\ ♀}$ the hybrid nature of the segmentation nucleus is easily recognized.

Baltzer, '09, in an extensive morphological study of the chromosomes of the European forms, *Strongylocentrotus lividus* and *Echinus microtuberculatus*, was able to identify single chromosomes which could be recognized in the hybrid $\frac{\textit{Strongylocentrotus}\ ♂}{\textit{Echinus}\ ♀}$.[1] The main facts which he describes may for the sake of comparison be briefly stated at this point.

The chromosomes of *Strongylocentrotus* are in general rod-shaped bodies which vary in length. The longest are over three times the length of the shortest. In addition to these there are in one-half of the eggs two and in the other half, three hook-shaped elements. The occasional third hook is smaller than the other two which are equal in size and evidently form a somatic pair. In all of the eggs the somatic number of chromosomes is the same; *i. e.*, thirty-six. In accordance with previous conclusions upon the occurrence of the heterochromosomes in insects, the dimorphism in the chromosome groups of *Strongylocentrotus* is correlated with the dimorphism of sex in echinoderms. However, Baltzer, from his observations on *Echinus* eggs cross-fertilized with *Strongylocentrotus* sperm, together with observations on multipolar spindles in disperm eggs, concludes that the odd hook is originally present in the egg. Instead of two kinds of sperm as, we know, exist in the insects, in the echinoderms there are two kinds of eggs. To quote from Wilson, '10, "it is the female that is the heterogametic sex while the male is homogametic. . . . Sex production in these animals must therefore conform to the formulas,

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[1] Boveri in his "Zellen Studien," Heft VI., 1907, Jena, called attention to Baltzer's observations which were being made at that time in Boveri's laboratory. He publishes one figure of *Strongylocentrotus* showing a hook-shaped chromosome. He himself in 1890 had noted the occurrence of rods of different lengths in certain echinoderms.

Egg F + Spermatozoan G = FG, Female.
Egg G + Spermatozoan G = GG, Male."

Tennent has obtained evidence which, I think, shows that these formulas do not hold for all echinoderms. An account of his interesting observations appears elsewhere in this journal.

In *Echinus*, which also contains thirty-six chromosomes, the occurrence of three characteristic pairs was established; viz., a pair of large hooks, a pair of small horse-shoe or V-shaped chromosomes and a pair of very long rods. A small V-shaped unpaired chromosome is also found in one half of the eggs which corresponds to the unpaired hook in *Strongylocentrotus*.

In a later paper, '10, Baltzer describes characteristic elements in the chromosome groups of *Sphærechinus granularis* and *Arbacia pustulosa*. The number of somatic chromosomes in *Sphærechinus* is forty-two. All are rod-shaped. Two somatic pairs could however be distinguished in all of the eggs by reason of their excessive lengths. No odd chromosome could be demonstrated in this species due, no doubt, to the lack of differentiation in form of the members of the group. *Arbacia* showed a number of small hooks and U-shaped chromosomes but the unfavorable nature of the egg prevented detailed study. The somatic number is forty. The chromosomes in this species resemble those of *Arbacia punctulata* figured by Tennent, '07, in that they are as a whole comparatively short.

In 1910, before the appearance of Baltzer's later paper, Miss Heffner published observations made in this laboratory upon *Toxopneustes variegatus*, one of the American species which has proved so valuable in cross breeding experiments.[1] She found in straight fertilized *Toxopneustes* eggs a somatic pair of long rods approximating in length the long rods of *Sphærechinus*. Half of the eggs contain two and half three V-shaped elements. The unpaired V is undoubtedly the accessory chromosome. The chromosome number in *Toxopneustes* is thirty-six, the same as in *Strongylocentrotus* and *Echinus*. Miss Heffner also examined *Arbacia punctulata* but found the eggs unfavorable for detailed cytological study, a fact which forms another point of resemblance between the American and European species described.

The present paper deals with two additional species of echinoids, *Hipponoë esculenta* (*Tripneustes esculentus*) and *Moira atropos*. My special object has been to determine whether any or all of the chromosomes exhibit any of the various expressions of individuality which have been reported for the other forms mentioned above.

I am indebted to Dr. Tennent, at whose instance the work was undertaken, for the material used and direction during the course of its investigation. I wish also to express my gratitude to Dr. Stevens for her interest and helpful suggestions.

Hipponoë esculenta.

The *Hipponoë* material was collected and preserved at the Tortugas laboratory of the Carnegie Institution in the summers of 1909 and 1910 by Dr. Tennent. It was prepared in the usual manner for study, the only stain used being Heidenhain's iron-hæmatoxylin. Observations made on first and second cleavage spindles form the chief basis of my conclusions.

The character of the *Hipponoë* spindle delayed somewhat the solution of the problem at hand. The cytoplasm of the egg is beautifully clear and the chromosomes compared to other echinoderm chromosomes are of good size, but during division they are so crowded on the spindle that they obscure one another. This may be attributed to several causes. It may be due to the thickness of the chromosomes which is a specific character. Figs. 1*a*, 1*b* and 25 show equatorial plates of *Hipponoë* and *Moira* respectively, and by comparing them one sees that the *Hipponoë* rods are thicker than those of *Moira*. It may be that the spindle radius is less in *Hipponoë* than in other forms. Again an attractive force may exist among the elements of one daughter group and this might cause clumping.

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[1] Tennent, '07, '10*a*, '10*b* and '11.

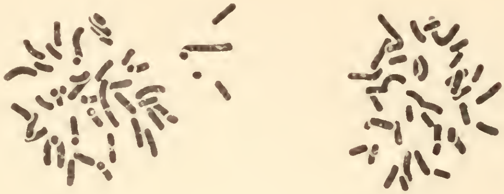
There are many reasons why the anaphase stages are most favorable for studies of this sort but there is no doubt that much can be gained from a study of equatorial plates. As will be seen from the figures referred to, the polar views of metaphase stages are especially suitable for determining the relative thickness of the chromosomes, although as Tennent, '07, has pointed out, the contrast is rarely great enough to justify the exclusive use of this character as a means of distinguishing between the chromosomes of two species in hybrids. The segments of the contracted spireme in metaphase have not yet been influenced by the spindle fibers or the force which produces division, whatever that may be, and therefore show a uniform thickness. Such equatorial plates are composed of rod-shaped chromosomes of varying lengths. They may be straight, curved or even bent at various angles. In the latter case it could not be determined whether the resulting V-like chromosomes were to be identified with the V's found in the division stages. Neither is it possible to determine the length or number of the rods. Crowding and overlapping in some parts of the plate prevents the separation of the elements or their correct measurement. If however the chromosomes maintain constant size relations, as we should expect from the abundance of evidence on this point gained from forms more suitable for its demonstration, and since, as I have shown in my figures, the thickness of the rods in this stage is constant, it seems that the relative lengths of the chromosomes at this stage must be constant also and that in the subsequent division stages where the thickness of the rods does evidently vary the length relations vary also. This I consider a point of great importance since it is on just such a comparison of the lengths of the chromosomes in division stages that Baltzer, '10, depends for much of his evidence as to the identity of the eliminated chromosomes in the hybrids. Baltzer makes no comparison between the equatorial plates of the species that he studied. In his figures of anaphases of different species the daughter chromosomes are shown as rods of equal thickness. He regards the apparent variations in the thickness of the rods in sections stained with Heidenhain's iron-hæmatoxylin as the fault of the stain and his conclusions as to the uniform thickness of the rods are drawn from observations made upon aceto-carmine preparations. One would naturally expect to find the rods which lie farthest from the surface of the section retaining the stain longest. The deeper lying rods might then appear thicker but all of the rods lying in one optical plane would show the same width. All of the rods in one optical plane, however, are not of the same width. It might be argued that a disturbance in the section after staining or variations in the thickness of the sections would produce this effect but the variations in width are of too frequent occurrence to be ascribed to any such causal factors.

I am convinced from my own observations that the rods of one spindle not only vary in thickness as they are drawn toward the poles but they vary irregularly. The explanation is simple. During division the proximal ends of the two halves of a rod are drawn toward opposite poles while the distal ends are still united. This latter opposing force results in the lengthening of the two daughter chromosomes during their separation. As soon as the separation is complete the rods begin to contract. Since some rods are much longer than others the accomplishment of their separation and their subsequent contraction would naturally be delayed and their relative lengths in a late anaphase would be more apparent than real.

For illustration I refer to Baltzer's later paper, '10, Figs. 1, *a* and *b*. In these he shows a *Strongylocentrotus* spindle with only two rod-shaped chromosomes over eight mm. long. The two spindles shown in his Figs. 4, *a* and *b*, and 12, *a* and *b* from the same species each show three rods which exceed eight mm. On the other hand Figs. 5, *a* and *b*, show thirteen and Figs. 6, *a* and *b*, ten chromosomes from one spindle which are more than eight mm. long.

Again Figs. 5, *a* and *b*, show one chromosome over 15 mm.

long while the next longest measures only a trifle over 11 mm. If as we would naturally suppose, the two longest in this case form a somatic pair, it is demonstrated that the force which causes stretching during division and contraction after the daughter elements have separated, may work very irregularly indeed and that the chromosomal lengths taken from these stages from a very unsafe basis for comparison. This is not meant to imply that length as a factor in identification is to be entirely eliminated.

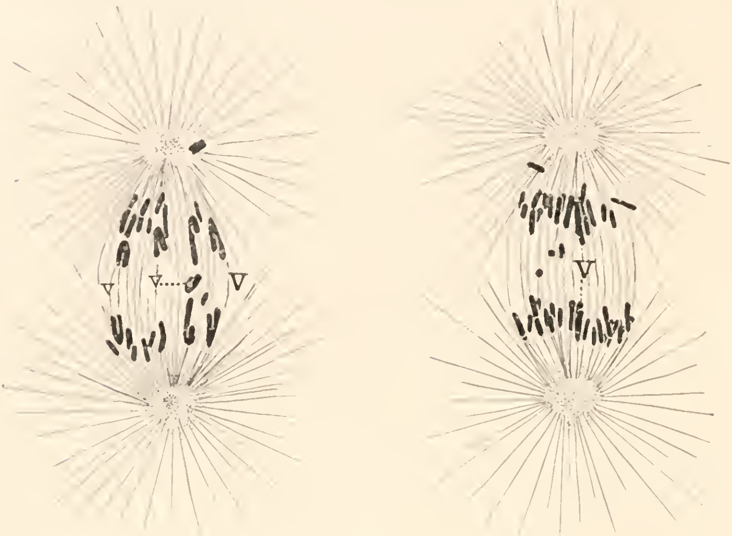

1 a 1 b

Figs. 1*a* and 1*b*. *Hipponoe*. Chromosomes of equatorial plates from the same egg, two-celled stage. Drawn at a magnification of 1,500 diameters.

In the later anaphases of *Hipponoe* three types of chromosomes appear. Commonest among these are the rod-shaped elements. As will be seen from Figs. 2*a*, 2*b*, 3*a*, 3*b*, 4*a* and 4*b*, none of these are of remarkable length. In Fig. 4*a* is given the only case that I found in which a long rod appeared. This unusual long chromosome would not resolve itself into two with the most careful focusing. Its isolated occurrence justifies the interpretation that it is an illustration of the tendency of *Hipponoe* chromosomes to clump together.

In none of my figures of *Hipponoe* are all of the rods shown. All attempts to separate the chromosomal complex into its units were unsuccessful. Figs. 2*a* and 2*b* show one spindle in which the chromosomes were unusually well separated. The variation in the thickness of the rods was reported by Heffner, '10, for *Toxopneustes*. Figs. 3*a* and 3*b* illustrate the same fact for *Hipponoe*. Accuracy is extremely difficult to achieve in the drawing of such minute objects as Echinoid chromosomes. All possible care was taken to indicate the true size relations.

Two somatic pairs of V's occur and these are shown in Figs. 5, 6, and 7 to be of two sizes. Their demonstration was not easy in the late division stages for as Miss Heffner found in *Toxopneustes*, the arms of the V's lie parallel. In *Hipponoë* one



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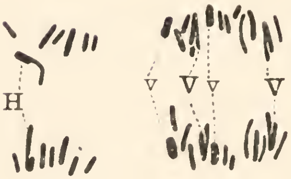

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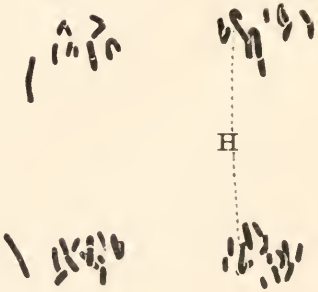


4 a

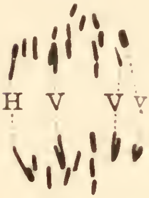
4 b

FIGS. *2a* and *2b*. *Hipponoë*. One spindle in anaphase from two sections. Two pairs of V's and a hook are present.

FIGS. *3a* and *3b*. *Hipponoë*. Same as Figs. *2a* and *2b*. *H*, the hook-shaped chromosome.

FIGS. *4a* and *4b*. *Hipponoë*. Late anaphase. Not all of the rod-shaped chromosomes were drawn in these figures. All drawings made at a magnification of 1,500 diameters.

meets with the additional difficulty caused by the crowding of the chromosomes. For their study early anaphases proved most favorable. A reconstruction from one of these is shown in Fig. 6 and the V's from one are drawn in Fig. 7. The somatic pairs in these figures are easily recognized in this stage from their size as well as from their behavior. In the case of the pair of larger



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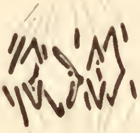

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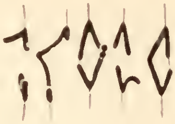

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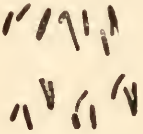

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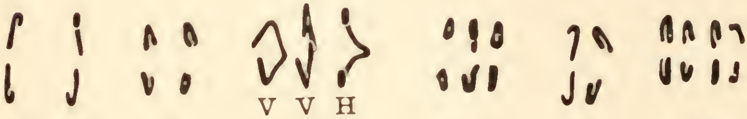

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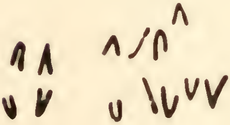
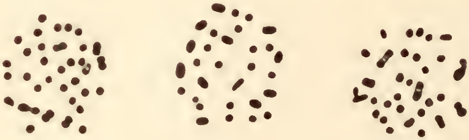


16 17



18a 18b 18c

FIG. 5. *Hipponoë.* Mid anaphase.

FIG. 6. *Hipponoë.* Reconstruction from an early anaphase showing the relative size of the V's and hook.

FIG. 7. *Hipponoë.* V's and hook from one spindle.

FIG. 8. *Hipponoë.* Section showing large V's and hook.

FIGS. 9 to 17 inclusive. *Hipponoë.* The two-armed elements from nine different eggs. These figures illustrate the comparative frequency with which the different elements are observed. The hook is the most conspicuous. Fig. 15 is from an egg which contained no hook.

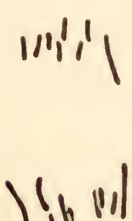
FIGS. 18*a*, 18*b* and 18*c*. *Hipponoë.* Polar views of daughter plates, 33, 30 and 32 chromosomes respectively. Magnification 1,500 diameters.

V's one arm of the V completes its division first in almost every instance. The question as to whether this is due to a mechanical hindrance in division or to a real difference in the size of the two arms has been discussed by Miss Heffner. I am inclined to the opinion that the two arms are of different lengths since that appearance predominates. Fig. 3*b* is a typical view. The larger pair is easily identified but one can be sure of only one smaller V. Its mate may be the small thick element, *v*, lying next to one of the larger V's. In Figs. 11, 12, 13, 15, 16 and 17 some of the V's from several other spindles are shown. Owing to individual differences in the eggs it would be impossible to identify the large or the small pair as such unless the other pair could be distinguished.

Miss Heffner did not observe any constant difference in size or behavior which might mark the unpaired V in *Toxopneustes*. The size relation of the *Hipponoë* V's is very plain in early anaphases as I have already explained.

I have examined sections of *Toxopneustes* eggs made from the same material that Miss Heffner used and am able to confirm her observations upon the appearance and behavior of the V-shaped elements. The long rods are also very conspicuous (Figs. 19, 20 and 21). A peculiarity, presumably of the egg, calls for different degrees of differentiation in staining early and late anaphases. I could not find on the slides that I examined early anaphases where the condition of things could be made out satisfactorily although there were plenty of later stages which were as clear as one could wish. In these I have observed and give figures (20 and 21) which show a difference in the size of the V's which would indicate that the unpaired V is the smallest, yet I hesitate to accept this alone as evidence that such is the case. Fig. 21 shows two large and one small *V* in the upper group. One large *V* is missing from the lower group. The indications were that it had been displaced by the knife in sectioning. Miss Heffner's Figs. 1*a*, and 3, *a* and *b*, depict two spindles in early anaphase. From these figures and from what I find in *Hipponoë* where such stages are comparatively abundant and convincing I feel justified in concluding that the unpaired V or heterochromosome in *Toxopneustes* is the smallest V of the complex.

In Figs. 3, 9 and 10 is shown the third type of echinoderm chromosome, the hook. It corresponds in all respects to the hook described by Baltzer. In *Hipponoë* it occurs singly and is not present in all of the eggs. Of twenty-nine eggs in which its presence or absence could be determined with certainty seventeen contained the hook. In late stages it is the most conspicuous element of the complex and is therefore quite easily detected when present; but when it is not found, one cannot always be sure that it is not hidden among the other chromosomes. In examining the eggs then more of those which lacked the hook would be rejected as positive evidence than of those in which it occurred, so I believe we are safe in asserting that half of the fertilized eggs contain the hook and in half it is absent. As Dr. Tennent, '11, this journal, has shown, the dimorphism in somatic chromosome groups of fertilized *Hipponoë* eggs can be associated only with a dimorphism in the sperm.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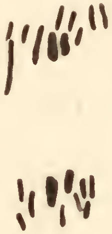
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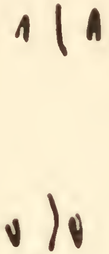
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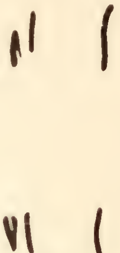
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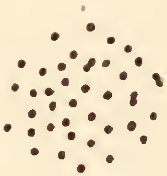
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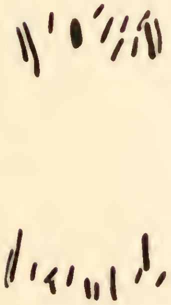
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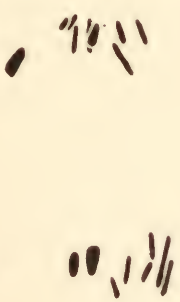
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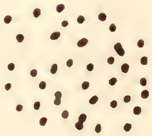
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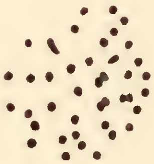
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FIGS. 19*a* and 19*b*. *Toxopneustes*. Late anaphase showing the long rods.

FIGS. 20*a* and 20*b*. *Toxopneustes*. Conspicuous chromosomes from a late anaphase.

FIGS. 21*a* and 21*b*. *Toxopneustes*. Late anaphase.

FIGS. 22, 23 and 24. *Toxopneustes*. Polar views of daughter plates, 38 chromosomes. Magnification 1,500 diameters.

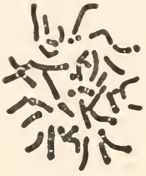
The decision having been reached as to the differential character of the hook-shaped chromosome, the question arose as to the nature of its morphological relation to the other chromosomes. All such elements of which we have microscopical proof, belong to two classes, the accessory or unpaired chromosomes and the paired heterochromosomes or idiochromosomes. This does not take into account those cases like *Culex* and *Theobaldia* where no differential element is visible in the sex cells. In both of these classes the germ cells of one sex lack a definite chromatin mass which is present in the other. The single or unequal pair of chromosomes of the one is replaced in the other by an equal pair both members of which correspond in size respectively to the *single element* or to the *larger element of the unequal pair*. In *Hipponoë* the search for evidence on this point revealed no pair of chromosomes equal to or exceeding the hook in size. This led to the conclusion that a corresponding pair of hooks does not exist. It was then thought that if such a pair was present its members were probably ordinary rods. No rods equal in size to the combined lengths of the two arms were present in any of the eggs. The question as to whether the hook was a multiple chromosome was considered. This probability which I will discuss later, suggested that perhaps the differential sex element consisted of only one arm of the hook. No rods equal in size to the long arm of the hook were discovered in any of the eggs. If such rods were present they might escape detection in many instances but it is improbable that they should be so consistently over-looked in all of the eggs. In Figs. 2*a* and 2*b* an attempt was made to draw all of the chromosomes from one spindle. The hook and V's are plainly visible. All of the elements shown could be separated by careful focusing but no rod longer than the arms of the large V's was observed. It appears highly probable that the long arm of the hook is unmated in somatic cells containing the hook and that no corresponding pair of long rods is present in cells which contain no hook. It would be impossible from this material to determine whether or not the short arm of the hook was mated. Many short rods approximating it in length occur but they could not be studied individually.

I have failed to determine the number of chromosomes in these eggs. Figs. 18*a*, 18*b* and 18*c* give some of the best polar views of daughter plates. Fig. 18*a* shows thirty-three bodies one of which appears as though it might consist of two or more chromosomes crowded together. I was not able as were both Heffner and Baltzer to identify with certainty the V's or hook in such views. As these observers have explained, the ordinary rods seen in polar views appear as small black dots. Two of these in contact are to be interpreted as the closely appressed arms of the V-shaped chromosome. Miss Heffner's figures of the polar views of daughter groups of *Toxopneustes* chromosomes are very clear and her interpretation of them is undoubtedly correct. Baltzer also finds the V's in polar views appearing as two closely set dots. In polar views of *Hipponoë* I have often observed what I at first supposed might be a V. Besides the small round dots typical of the rod-shaped chromosomes, one finds in every plate a number of elliptical bodies of twice the size of the ordinary dots. These one naturally interprets as the two arms of a V with their adjacent surfaces flattened together. In some cases a very evident constriction coincident with their narrowest diameter makes such an interpretation imperative. This constriction is not however evident in the majority of cases and very often more of these elliptical bodies appear than are called for by the number of two-armed elements in *Hipponoë* (Fig. 18*b*). In all cases then the question is still open as to whether these are V's or two rod-shaped individuals or even, as is quite possible, only one rod viewed obliquely. The latter possibility in addition to the difficulty presented by untranslatable amorphous chromatin bodies in these daughter plates makes an accurate count impossible. The number counted most frequently was thirty-two but in no case could I feel sure that the number counted was correct.

Moira atropos.

The egg of *Moira* is much better suited to detailed study than the egg of *Hipponoë*. The tendency of the chromosomes to crowd together is not so prevalent. Although my material was very limited and contained comparatively few spindles some general facts were gathered which it may be worth while to record.

Fig. 25 is a polar view of the equatorial plate of the first segmentation nucleus. As already stated the chromosomes are more slender than those of *Hipponoë*. Fig. 26 is a section through a metakinesis stage. Only a few of the chromosomes are shown. Three types appear; the ordinary rods, two small V's and two club-shaped elements. In Figs. 27 and 28, which are analytical drawings of two early *Moira* spindles, the same three types appear. It could not be determined whether the club-shaped chromosomes were small hooks or not. The chromosome X in each of the figures mentioned above has the appearance often presented by the *Hipponoë* hook. The small V's are numerous but their number is not evident. Although the *Moira* chromosomes are comparatively well separated on the spindle the fact that the number is not the same in all of the figures is evidence that even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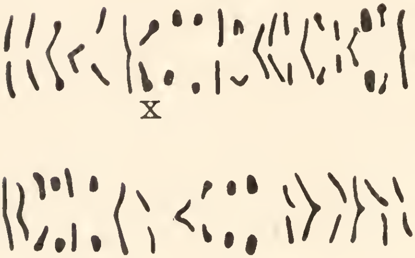

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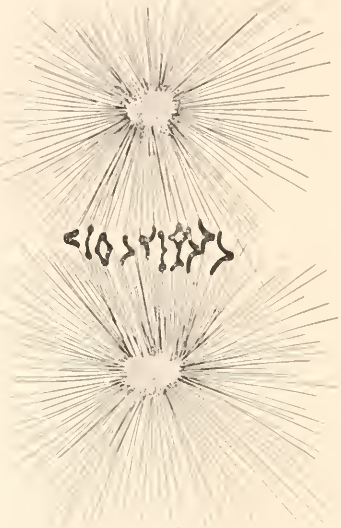
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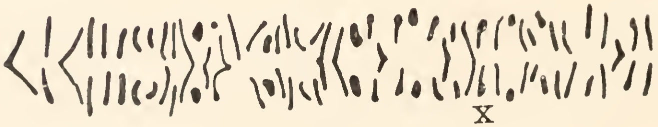

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FIG. 25. *Moira*. Equatorial plate from first segmentation nucleus.
FIG. 26. *Moira*. Beginning anaphase.
FIG. 27. *Moira*. Chromosomes from an early anaphase. *X*, hook?
FIG. 28. *Moira*. Same as 27. Magnification, 1,500 diameters.

here mistakes in drawing are almost inevitable. It may be that some shown are really two chromosomes so close together that they could not be separated with the magnification used. The series is also taken from two or more sections and there is the ever present possibility that some may have been displaced. Figs. 29*a* and 29*b* show a later anaphase of which Fig. 30 is a detailed drawing. A hook (*x*) is shown to be present, but many more observations would be necessary to demonstrate its presence beyond a doubt. The club-shaped type of chromosome is not in evidence here.

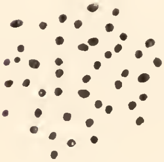
As was to be expected from lateral views of anaphases the only daughter plates I found were especially adapted to an exact enumeration of their elements. The number is shown in Figs. 31*a* and 31*b*, which are daughter plates of the same spindle, to be forty-six, the largest number yet reported for the echinoderms. Fig. 31*b* was drawn from two sections. The character of the *Moira* spindle is such that I consider the evidence as to the number of chromosomes gained from this one plate much more convincing than all of the combined evidence of a like sort which I obtained from *Hipponoe*. I hope to extend the observations on this species later.

The *Moira* material was collected at Beaufort, N. C., in 1907. Its treatment was identical with that of *Hipponoe*.

DISCUSSION.

From conclusions reached by Baltzer, '09, and Tennent, '11, it seems probable that in different species of a limited group the heterochromosome may originate in different sexes. Both of these authors used the same method of investigation so that the evidence of one bearing on this point possesses no peculiar advantage over that of the other. Both are equally justified in their conclusions. In *Strongylocentrotus lividus* and *Echinus microtuberculatus*, it is the female that is morphologically the heterogametic sex while the male is in the same sense homogametic. In the light of our present knowledge of the occurrence of the accessory chromosome in other groups these facts present an interesting anomaly.

If as Baltzer found in *Strongylocentrotus* and as Miss Heffner found in *Toxopneustes* the somatic number of chromosomes is an even number in both sexes we must conclude that the odd chromosome is the eccentric member of an unequal pair. This of course implies the assumption that in the odd chromosome we are dealing with a univalent and not a plurivalent element. The number of chromosomes in *Hipponoë* may or may not be even. In the five species of sea-urchins already investigated the somatic number has been reported as even. The conclusions have however been drawn from averages of many counts. Since the counts of single plates varied an exact statement as to whether the diploid number of chromosomes is the same and even in both sexes, or differs, being even in one and odd in the other, seems hardly justifiable.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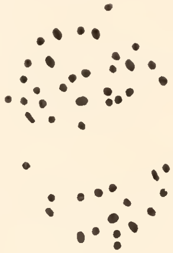
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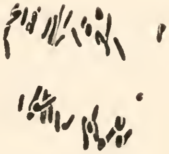
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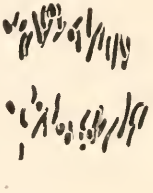
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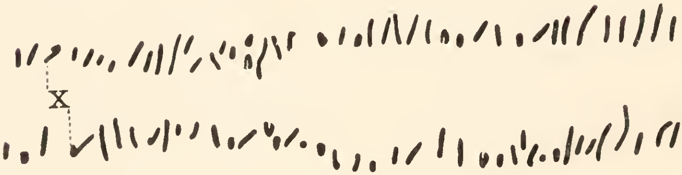

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FIGS. 29*a* and 29*b*. *Moira*. Anaphase from two sections.
FIG. 30. *Moira*. Chromosomes from Fig. 29. *X*, hook?
FIGS. 31*a* and 31*b*. *Moira*. Polar views of two daughter plates from the same spindle. 46 chromosomes. Fig. 31*b* is from two sections. 1,500 diameters.

In comparing daughter plates of *Hipponoë* with those of *Toxopneustes* I found as a rule that the chromosomes in *Toxopneustes* were better separated. Even there the majority of counts could not be made with any assurance of accuracy. Miss Heffner reported thirty-six chromosomes for *Toxopneustes*. I found four plates which showed thirty-eight without any doubt. Three of them are shown in Figs. 22, 23 and 24. I also counted thirty-seven in two very clear daughter plates of the same cell.[1] I made a few counts where thirty-six seemed to be the number but in these cases I was not sure that I had counted correctly. Other counts were made but they were equally valueless. The *Toxopneustes* material was too limited to permit definite conclusions as to the actual numbers. I am convinced that more than thirty-six chromosomes occur in some eggs. An actual determination is difficult but not impossible. Averages in a case of this sort are useless.

Whatever the morphological relations of the hook to the other members of the complex, their consideration leads us to a recognition of possible existence of further anomalous conditions in this species. All unequal pairs of heterochromosomes or unpaired heterochromosomes of previous observation find a place in the series postulated by Stevens, '11, which, beginning with *Culex* and *Theobaldia*, where no heterochromosome occurs, includes all of the various unequal pairs and ends with forms containing an odd or unpaired chromosome. We may imagine all of the members in this series derived from forms analogous to *Culex* by the gradual diminution and final elimination of one chromosome from a somatic pair. Assuming that the hook in *Hipponoë* is the morphological equivalent of the odd chromosome or the larger member of an unequal pair it follows that this element cannot be accounted for in this manner. On the contrary we must conceive of the change from the *Culex* heterochromosome type to the *Hipponoe* type to have come about by an increase in the size of one member of a somatic pair. It is quite as reasonable to suppose that the evolutionary processes within the nucleus involve an increase in the amount of chromatin as it is to assume that they are accompanied by its elimination. In support of this view we have the fact that the hook is the largest element in the *Hipponoë* complex.

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[1] Prof. E. B. Wilson, in *Arch. f. Entw. d. Organismen v. Roux.*, Bd. XII., Taf. XVI., Fig. 43, gives a daughter plate of *Toxopneustes* showing 37 chromosomes. The explanation of the figure states that the number is 36. The coincidence is interesting in this connection. In earlier papers he raised the question as to whether the number was 36 or 38.

Baltzer has suggested that the hook is a plurivalent element composed of two chromosomes united at their polar ends. Admitting such an interpretation it still remains probable that *Hipponoë* cannot be included in the above series since it was impossible to find for the long arm of the hook of some eggs a corresponding pair of equal rods in others. The long rods of *Toxopneustes* are so conspicuous in late anaphases that it seems as though the presence of a similar pair in *Hipponoë* could not be a matter of dispute. Figs. 19, 20 and 21 show anaphases of *Toxopneustes* containing the characteristic long rods. These *Toxopneustes* rods are slightly longer than the long arm of the hook and the groups to which they belong are perhaps not so compact as daughter groups in *Hipponoë.*

With Baltzer's suggestion under consideration the question arises as to whether the V's which resemble the hook in all respects but length of arms are also to be regarded as multiple chromosomes. If so the method of grouping in *Hipponoë* differs from anything previously described.

The idea that the heterochromosome is associated with the inheritance of sex is a point of common consideration when treating of this differential element.

From the experimental evidence as to the inheritance of sex Castle, '09, has concluded that femaleness depends upon the presence of some factor wanting in the male, the differential factor. Two classes of female zygotes are recognized in Castle's scheme, one, class A in which "femaleness is attained only when the differential factor is doubly represented in the individual" and another, class B, in which femaleness is attained whenever the differential factor is present in one only of the conjugating gametes which produce the individual." The former for which Wilson gives the sex formulas; XX = female and X = male, X in this case designating the differential factor, is represented by the insects. Castle says, "Direct cytological evidence of the existence of class B is not known at present." The formulas, XO = female and O = male embody Castle's idea.

Hipponoë falls into class A only upon the assumption that the hook is a multiple chromosome, the long arm being the differential element, and that there exists in one-half of the fertilized eggs a pair of rods the same length as the long arm. There is no observational basis as yet for either of these assumptions. Neither does our evidence place *Hipponoë* in class B since Professor Tennent's hybridization experiments show that the hook must come from the male. If however the hook is not a multiple chromosome the case is analogous to class B, the sex formulas being reversed to indicate that the male is a heterozygous dominant and the female a pure recessive, XO = ♂ and OO = ♀. The conclusion is the same if we identify the differential element with the long arm of the hook and assume that none of the eggs contain a corresponding pair of long rods.

Clearly our conclusions as to the composition of the hook and its meaning must be deferred until our knowledge has been increased by further evidence gained from this and other species. McClung's views upon the taxonomic value of the chromosomal complex suggest that the study of other species of the same genus would prove most profitable. From a comparison of the two species of the genus *Arbacia* described by Tennent and Baltzer one finds similarities in the chromosomal groups which lend significance to this suggestion. Broader cytological study of the echinoderms is also necessary in order to discover the fundamental principle by which we can reconcile such diverse phenomena as those presented by *Strongylocentrotus* and *Echinus* on the one hand and *Hipponoe* on the other. The difficulties which attend the study of echinoderm chromosomes makes it desirable to extend the number of workers in this field.

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BIOLOGICAL BULLETIN

A STUDY OF THE RELATION OF TISSUE DIFFERENTIATION TO RATE OF GROWTH DURING REGENERATION.[1]

W. F. ALLEN.

ACKNOWLEDGMENTS.

This work was done under the direction of Dr. Chas. Zeleny, whose advice and criticism have been invaluable. I am also indebted to Professor H. B. Ward for suggestions and encouragement, to Mr. S. F. Prince for help with the drawings, to Mr. W. Scott, of Indiana University, for *Amblystoma* material efficiently supplied and to the Illinois State Laboratory of Natural History for the loan of valuable literature.

OBJECT.

The object of this study was to find whether the greatest speed of regeneration occurs before or after or coincident with the completion of tissue differentiation. If the same correlation holds that was noted by Minot (1908) in ordinary growth, we should expect to find the rate of growth decreasing after the major tissues have been well developed and still more so after differentiation is essentially complete. In the hope of securing definite information the present effort has been made to secure data in that definite line, using two distinct types for study.

EXPERIMENT I. A MATURE ANIMAL.

THE OLIGOCHÆTE WORM, *Limnodrilus claparedianus*, Ratzel.

Material and Methods.

The work was begun in early October, 1910. At that time there was not much choice of material for such investigation as a preliminary survey of the local field soon showed. Lack of better material was therefore the principal reason for choosing *Limnodrilus*. Of points distinctly in its favor, the most prominent were accessibility, hardiness, adaptability to laboratory conditions and rapidity of regeneration. On the other hand, the small size, irritability, quickness and sandy food caused some serious difficulties. The specimens used were taken from a sandy slough margin just north of Urbana Fair Grounds. All were in good condition and in a few hours appeared quite at home under laboratory conditions.

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[1] Contributions from the Zoölogical Laboratory, University of Illinois, under the direction of Henry B. Ward, No. 9.

The effort was made to select 120 worms of equal median size, but their activity was so great and their appearance changed so quickly that the results showed considerable variation. Great care was taken to make exact transverse cuts at the middle, but here again, activity interfered and there was a considerable percentage of errors in cutting. For operation each worm was placed on a paraffine block and a quick even cut was made with a sharp, thin scalpel. Anterior parts only were retained. Those of the first five worms bisected were at once stupefied in weak chloretone solution and killed in Gilson's sublimate mixture (Lee, 1900). All other worms were handled in the same way. The second five was kept alive 1 hour after operation. All others were placed in 10 cm. Petri dishes in which was about 4 c.c. of sterilized native mud with 30 c.c. of tap water. Ten were placed in each dish except by miscount due to disappearance in the mud. Two dishes with unoperated worms were kept for check. One hundred were bisected. Water was changed in the Petri dishes twice daily immediately after taking the temperature of the water already in the dishes. The highest temperature recorded was 28° C., the lowest 21° C. The greatest fluctuation in 12 hours was 5°. While it was unfortunate that laboratory conditions were not such as to permit more uniform temperature, it is hardly probable that even this variation seriously affected these hardy worms, none of which died. Light and other conditions were kept as nearly uniform as possible. Direct sunlight was avoided altogether.

As noted before the first five was killed immediately after operation, the second in 1 hour, third in 6 hours, fourth in 12

TABLE I.

Limnodrilus.

	Individual Worm Number.	Length of Single Old Segment in mms.	Length of Whole Regenerated Part in mms.
1st day........	*7a*	.418	.114
	7b	.432	.057
	7c	.425	.114
	7d	.432	.076
	7e	.342	.038
2d day.......	*7f*	.418	.083
	7g	.380	(.034)
	7h	.361	.285
	7i	.380	.380
	7j	.418	.190
3d day........	*8a*	.342	.342
	9a	.494	.228
	9b	.304	.300
	10a	.380	(.114)
	10b	.418	.234
4th day.......	*8c*	.418	.608
	8d	.456	.380
	9c	.342	.608
	9d	.430	.684
	10c	.418	.284
	10d	.418	.608
5th day.......	*8e*	.418	1.254
	8f	.266	.988
	9e	.304	.722
	10e	.380	.950
	10f	.370	.912
6th day.......	*8g*	.456	1.824
	9g	.418	1.824
	9h	.418	2.012
	10g	.342	1.824
	10h	.418	2.204
7th day.....	*8i*	.284	3.648
	8j	.456	3.800
	8k	.418	(1.634)
	9i	.322	2.546
	9j	.304	1.900
	10i	.418	(.988)
	10j	.380	2.888
	10k	.380	3.268
	10l	.494	4.180
	10m	.380	(.076)
8th day.......	*11a*	.456	3.800
	11b	.342	4.788
	12a	.456	4.066
	13a	.418	5.928
	14a	.456	5.016

Explanation of Table I.—Table I. is a copy of the records, in millimeters, from which the growth rate for the total regenerated part was calculated. Numbers omitted because of undue influence upon the average are placed in parenthesis.

TABLE I.—*Continued.*

Limnodrilus.

	Individual Worm Number.	Length of Single Old Segment in mms.	Length of Whole Regenerated Part in mms.
9th day	11*c*	.456	7.448
	11*d*	.456	5.016
	12*b*	.380	7.400
	13*b*	.494	8.132
	14*b*	.456	6.840
10th day	11*e*	.342	7.400
	12*c*	.456	7.068
	12*d*	.532	(5.928)
	13*c*	.418	7.980
	14*c*	.380	8.626
11th day	11*f*	.418	6.346
	12*e*	.456	9.196
	12*f*	.456	7.670
	13*d*	.494	10.754
	14*d*	.361	7.752
12th day	11*g*		9.1
	12*g*		9.4
	13*e*		9.
	13*f*		9.6
	14*e*		9.8
15th day	11*h*		12.7
	12*h*		10.9
	13*g*		13.3
	13*h*		11.5
	14*f*		18.9
18th day	11*i*		10.9
	12*i*		17.2
	13*i*		16.8
	14*g*		16.3
	14*h*		13.7

hours and fifth in 24 hours. After that a group of five was taken each day to the twelfth day, the remainder on the fifteenth and eighteenth days. Since the specimens had to be killed for tissue study it was necessary to calculate the rate of growth from the condition in successive groups after killing instead of using periodic measurements of living worms.

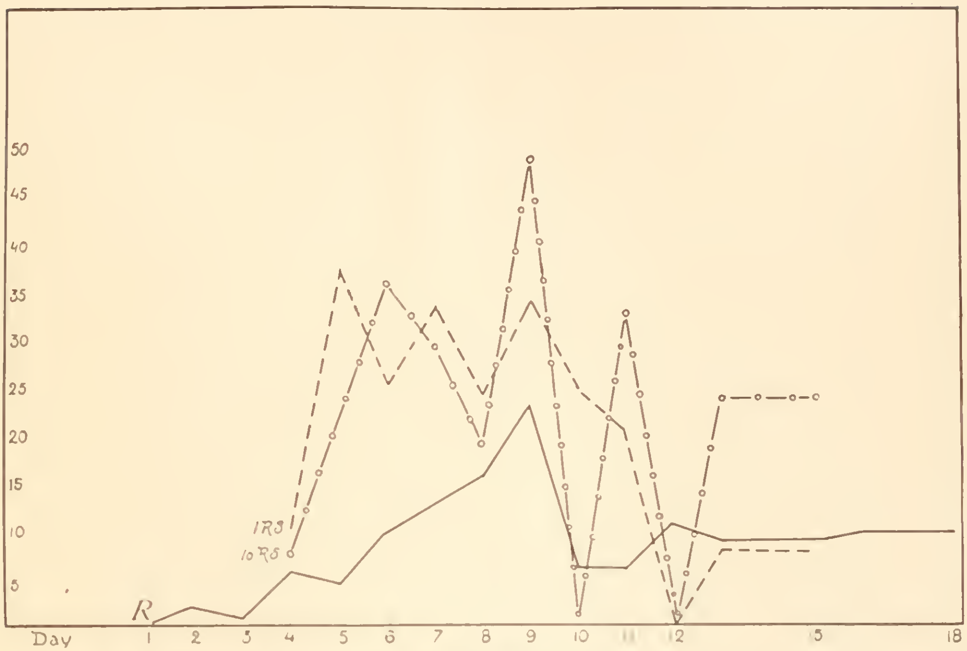
DATA.

The following sets of measurements were taken:

1. The total length of the regenerated part was measured by eyepiece micrometer under low power as the specimens lay in

cedar oil in a watch glass. (Table I. and Chart 1.) Crooked specimens caused some inaccuracy here.

Chart I.



Explanation of Chart I.—A graphic representation of the records contained in Tables I. and II. The continuous line *R* is for the total regenerated part. The broken lines (1 *RS* and 10 *RS*) are for the first and tenth regenerated segments respectively. Each 1/20 inch rise in the polygon represents an increase in rate of growth of 0.1 mm. for *R* and 0.01 for the 1 *RS* and 10 *RS*.

2. At the same time and under the same conditions an old segment, about tenth from the level of cut, was measured in order to get a basis of comparison of general results. (Table I.)

3. Measurements were made after mounting on slides, some after sectioning but most in cedar oil. In this way some of the errors due to crookedness were detected though the general result is not essentially different. (Table II.)

4. Every tenth segment of the regenerated part was measured for length. (Table II.)

As these measurements form the only basis for the construction of a polygon (Chart I.) to graphically illustrate the rate of growth it is worth while to give them a little general discussion. There are sources of error, which being well recognized, have been checked upon and guarded against as much as possible. (1) The measurements were made and the segments counted without the use of a mechanical stage. Since the specimens possessed very few good guide marks there may have been slight errors due to this cause. (2) Only one count was made in simpler cases although several counts were made in doubtful cases. (3) The correct measurements may be misleading at times because of variability in the speed of regeneration due to lack of uniformity in the bisecting cuts. This is due largely to the smallness and activity of the worms which made it impossible to cut precisely across or at the same level in the segments. Thus some cuts fell on the inter-segmental line, others at various distances within the segment, and several were diagonal. The

TABLE II.

Limnodrilus.

	4th Day.	5th Day.	6th Day.	7th Day.	8th Day.	9th Day.	10th Day.	11th Day.	12th Day.	15th Day.	18th Day.
R	.625	.92	2.15	3.06	5.26	6.94	7.09	8.38	9.29	14.29	16.43
1*RS*	.031	.068	.093	.126	.150	.184	.213	.234	.212	.258	.221
10*RS*	.015	.034	.070	.099	.118	.167	.168	.201	.192	.273	.246
20*RS*	.003	.018	.047	.073	.108	.141	.145	.165	.163	.247	.227
30*RS*	.002	.008	.022	.043	.101	.112	.122	.110	.142	.207	.197
40*RS*			.011	.020	.058	.083	.097	.102	.123	.179	.174
50*RS*			.008	.014	.037	.048	.068	.072	.092	.159	.164
60*RS*				.008	.017	.025	.021	.052	.076	.109	.145
70*RS*					.008	.011	.012	.021	.037	.102	.143
80*RS*						.007		.014	.015	.052	.115
90*RS*								.008	.012	.012	.074

A copy of the averages from which the growth rate for the first and tenth regenerated segments was calculated. A partial revision of the averages for the total regenerated part is also included here. Measurements are in millimeters. *R* = length of regenerated tissue. 1*RS*, 10*RS*, etc., first, tenth, etc., regenerated segments.

Explanation of Table II.—Table II. is a copy of the averages, in millimeters, from which the growth rate for the first and tenth regenerated segments was calculated. A partly revised list of the averages for the total regenerated part is also included here. A comparison of Tables I. and II., especially considering the measurements of old segments in Table I., will give some indication of one disturbing factor in the polygon, *i. e.*, varying sizes or extension of the worms at the time of killing. *R* = total regenerated tissue; 1 *RS*, 10 *RS*, etc., = first, tenth, etc., regenerated segments.

TABLE III.

Limnodrilus.

Day.	Epidermis.	Intestinal Wall.	Septa.	Muscle.	Nerve Cord.	Peritoneum.	Blood Vessels.	Chlorogogue.	Setæ.	Nephridia.
1	Old cells cover wound	Proctodeum well formed. Chaotic.								
2	Proliferating.	Proliferating	Column forming	Forming						
3	Proliferating.	Proliferating	Column formed.	Distinct	Anlage distinct	Forming				
4	Proliferating.	Little proliferation.	Well formed.	Distinct	Distinct.	Forming.	Ventral present.			
5	Distinct, thin cuticle.	Little proliferation.	Well formed	Distinct.	Well formed.	Well developed.	Ventral present.		Embryonic seta sacs distinct.	
6	Some mature.	Almost normal.	Well formed.	Distinct.	Well formed.	Well developed.	Both developed.	Present. Layer thin.	Embryonic seta sacs distinct.	
7	Some mature.	Almost normal.	Mature.	Mature.	Almost mature.	Mature.	Both developed.	Present. Layer thin.	Setæ well developed.	Anlage present.
8	Some mature.	Almost normal.	Mature.	Mature.	Almost mature.	Mature.	Both developed.	Present. Layer thin.	Setæ well developed.	One loop developing.
9	Some mature.	Mature.	Mature.	Mature.	Mature.	Mature.	Mature.	Present. Layer thin.	Nearly mature.	Immature.
10	Some mature.	Mature.	Mature	Mature.	Mature.	Mature.	Mature.	Present. Layer thin.	Mature.	Immature.

last point seems to be especially important as Müller (1908) and others have shown that diagonal cuts produce marked differences in the detail of regeneration. (4) The state of contraction at time of killing was also probably somewhat variable so that detailed comparisons would have to be made with caution. The effort was made to test this factor by measuring the regenerated parts with the old segment as a unit. This indicates greatest speed of regeneration on the tenth day instead of the ninth as indicated by the other method. (5) The small number of specimens available gives opportunity for undue prominence in the showing of individuals. Evident extremes were omitted from the averages. But, after all, the general effect of most of these factors would be to diffuse rather than to accentuate the polygon, and there seems to be ample warrant for saying that the greatest speed of regeneration is at the ninth or tenth day, which is near enough for present purposes. In close approximation to these results we have those from some unpublished work by Mr. Frank L. Pinckney, using *Limnodrilus* in this laboratory in the spring of 1910. As he used the method of successive measurements of living animals, the similarity seems to be practically conclusive.

In examining the polygons two striking points appear which are rather difficult to explain. In the first place, there is an apparent slackening in growth almost to the zero point on the tenth or eleventh day, the most obvious explanation for which seems to be in an accidental assemblage of extreme characters. At any rate there was no change in temperature or other observable laboratory conditions at that time that could possibly account for it. In the second place there is a rise in the later portion of the polygon. This, however, in the total regeneration seems to be due to the fact that there are still some 50 or 60 very young segments, many of which are just at their maximum, thus serving to largely balance the decline of the older regenerated segments, which is not in itself, so very great by that time. As the study of tissue differentiation has been made principally on regenerated segment no. 10, the polygons for segments no. 1 and no. 10 are shown (Chart I.). The maximum for no. 10 appears here at the ninth day but is apparently only more or less fortuitously coincident with the general maximum. Coincidence of this sort is hardly to be expected in all annelids. The abrupt rise for the tenth regenerated segment after the twelfth day is evidently due to accidental conditions in the groups.

For the differentiation study, longitudinal sections were used almost exclusively. All of these were sagittal or nearly so. While cross sections would certainly have given better histological detail they would have made it very difficult to interpret conditions in successive segments. The number of specimens favorable for sectioning was too small to allow the use of both kinds. The general features of the rate of differentiation in the regenerating part may be readily grasped by reference to Table III. The crookedness of some of the worms and the small size of all made interpretation of the sections rather difficult since so few sections in each specimen could be even approximately radial. There was the further difficulty, with many, of the fine sand in the digestive tract scratching and clouding the section even when it did not tear it. In spite of these disadvantages the characteristics of most tissues showed pretty well their relations to the embryonic and mature conditions. Ten tissues and organs are fairly easily distinguished in the mid body region of the normal worm. These will now be considered seriatim.

Mature epidermis is one layer in thickness with the form of cell varying from flat to cuboidal according to state of extension (Fig. 5). There is a well-developed cuticle. In regeneration there is first an extreme extension of epidermal cells in the neighborhood of the injury which in a few hours results in a covering of the cut surface. This extension is carried so far that the cells are quite thin and almost separated from each other. Then follows a period of very rapid proliferation in which the cells are three or four deep (Fig. 1). These cells are quite small, rounded, irregularly arranged and they have very little cytoplasm. This condition gradually changes until a portion four days old shows only a single layer. The cells even then are without well-marked walls, their nuclei are still prominent and they lack the definiteness so characteristic of the mature epidermal cell. A cuticle is easily distinguished by the fifth day and a sixth day epidermis is essentially mature though still staining a little differently and lacking some indefinable quality of the old tissue (cf. Figs. 1–5).

Much the same things may be said about the intestinal epithelium. In this tissue cilia are well developed on the third day and it is evidently functional by that time but it retains some slight embryonic features to about the eighth or ninth day.

Septa are first distinguishable in the embryonic mass as radial columns of cells (Fig. 1) extending partly across the cœlomic space. They rapidly fuse into a continuous membrane reaching evident maturity by the fifth day (cf. Figs. 1–6).

The longitudinal muscle layer of the body is first noticeable as a very thin, somewhat scattering layer of long, spindle-shaped cells just inside the epidermis (Fig. 1). It does not change very definitely except for increase of thickness and compactness. Although otherwise well developed by the seventh day at latest, it is not of typical thickness until later (cf. Figs. 1–5).

The nerve cord is recognizable on the fourth day and almost mature on the fifth. It is apparently typical by the seventh day. Note in Fig. 5 the ventral localization of nerve cells in the cord so characteristic in this group of worms (cf. Figs. 3–5).

The embryonic peritoneum seems recognizable by location on the third day but is well developed by the fifth (Fig. 5), and in evident maturity by the seventh (Fig. 4).

The blood vessels were not easily followed. The ventral vessel is unquestionably well formed by the fourth day. Both were observed in some specimens of the sixth and both are certainly mature by the ninth (cf. Figs. 3–5).

The study of chlorogogue was very unsatisfactory. It would seem that it should show well soon after maturity of the intestinal wall at least and it is distinguishable on the sixth day and thereafter, but no specimen was found showing it in typical condition even at the tenth day. It is possible that the sand in the intestine affected its preservation more than that of the other tissues.

The setæ are easily followed even in toto mounts. The embryonic seta sacs are well formed on the fifth day, and the setæ with all their muscles seem to be functional by the seventh. There is no question of their maturity by the 10th day (Fig. 4).

Nephridia were rather difficult to identify. An irregular mass of embryonic nephridial cells was first located, mainly by position, on the seventh day. A single loop, probably functional, is plain on the eighth (Fig. 4), but the organ does not appear to be mature even on the tenth.

All organs and tissues except the nephridia appear to be functional on the eighth day. They have also reached apparent maturity by the tenth day, some before. The data are not as complete as could be wished but they certainly are very indicative of a correlation between the maximum rate of growth and the period of distinct differentiation. This is especially true when we consider the fact that the tenth regenerated segment is the one for which these data are recorded and that its maximum speed of growth falls on the ninth day.

Experiment II. An Immature Animal. *Amblystoma* Tadpole.

Material and Methods.

Eggs laid near Bloomington, Ind., probably in the night of January 29 were received in good condition at this laboratory on February 1. They were kept in small lots under similar conditions except variation in water, until it was found that they did very well in tap water, after which it was used regularly. Up to the time of hatching, the main efforts for control were in the line of change of water, keeping temperature below 20° C., and avoidance of direct sunlight.

On February 10, 100 tadpoles were selected from a quite uniform lot and placed singly in berry dishes with about 50 c.c. of tap water. On the average, these individuals were about two days from the egg, none more than four nor less than one. The dishes were partly covered to reduce evaporation. Water was changed and the dishes washed two or three times per week. Fresh water was frequently added between times if evaporation seemed excessive. The temperature record was taken twice daily. No efforts to feed were successful until February 20. Within a few days many were feeding readily on live *Limnodrilus* which was used continuously to the end of the experiment.

By March 11 several deaths had occurred but the larger number of tadpoles seemed to be well adjusted, seventy of the better specimens averaging about 20 mm. in length were retained,

TABLE IV.

Amblystoma Tadpole.

	Individual Number.	Mm. Removed.	Mm. Regenerated.
Immediate	70	5.75	
	69	6.	
	68	5.5	
	67	5.5	
1st hour	66	5.25	
	65	5.	
	64	6.5	
6th hour	63	7.	
	62	7.	
	61	7.	
1st day	15	6.	
	45	7.5	
	46	6.5	
2d day	16	6.	.152
	2	7.5	.152
	59	6.	.19
3d day	29	7.	.38
	14	5.5	.38
	32	6.75	.35
4th day	17	6.	.45
	47	7.	.76
	44	8.5	.91
5th day	28	6.5	1.14
	58	7.	died
	3	6.	1.03
6th day[1]	33	7.	(.38)
	48	5.5	1.71[1]
	13	7.5	2.43[1]
7th day	43	7.25	1.90
	57	5.25	2.05
	18	6.5	1.37
8th day	27	6.5	2.28
	4	4.25	2.05
	19	6.	1.97
9th day	49	6.	2.28
	12	7.5	2.13
	26	7.	2.20
10th day	56	6.75	3.75
	5	6.	3.25
	35	6.	2.25
11th day	6	6.25	3.25
	41	7.	3.
	11	7.	3.75

TABLE IV.—*Continued.*

Amblystoma Tadpole.

	Individual Number.	Mm. Removed.	Mm. Regenerated.
12th day	55	7.5	3.5
	51	6.75	4.
	36	7.	4.
13th day	21	7.5	4.
	25	9.	4.
	24	7.	4.
14th day	54	7.5	4.5
	7	6.25	4.75
	37	7.	4.25
15th day	22	7.	5.
	39	6.25	5.
	38	6.	5.
16th day	8	7.	4.25
	23	4.5	3.5
	53	6.	4.5

[1] Killed five hours later. Deduct 20 per cent.

the others discarded. Operation was performed on March 16. Ten specimens were kept for check unoperated. Sixty had approximately one-half the tail removed. The anal opening and the tip of tail were taken as guide marks and the effort was made to cut one-half way between on estimate. It was also intended that the cut should be a right angles to the notochord. Of course, there were errors in both ways, some of the first being partly shown by the measurements in Table IV. The cut was made with a botanical razor against a block of paraffine. In order to steady the razor it was held at each end, the arms being rested on a table and the fingers on the paraffine block. Some of the error in judgment was due to some tails being more attenuate at tip than others.

Out of the 60 operated upon, four were killed immediately, three in one hour and three in six hours. The others were killed in groups of 3 on successive days from first to sixteenth inclusive. One of the three was taken from one end of the table the others from the other in order to avoid any possible influence of position. Gilson's mixture (Lee 1900) was used for killing followed by 70 per cent. alcohol, iodised 85 per cent. and the usual after procedure. Delafield's hæmatoxylin followed by picric acid gave excellent results in staining, the muscles showing especially well.

Two adverse conditions must be kept in mind throughout the consideration of this experiment. (1) Lack of ability to control temperature evidently affected the rate of growth seriously as indicated by Chart II. (2) The old tissue is itself so embryonic in character that it is extremely difficult to make an accurate measurement of the amount regenerated or an adequate description of its changing features. Some of the vagaries of the polygon must be attributed to both these causes. General conditions were good as is shown by the fact of only one death in the regular series. Drawings are omitted because the requisite series is too complicated for a paper of this character.

DATA.

These descriptions are somewhat indefinite because the facts themselves with few exceptions lack clear definition.

By the end of the first hour the epidermis had stretched over and very nearly covered the cut surface. In six hours it showed rapid proliferation, with cells closely and irregularly massed and three or four deep. This condition remained much the same in general appearance except for outward extension of the entire mass at the end of the first day. In two days the older regenerated epidermis was indistinguishable from the neighboring primary epidermis, though still proliferating at the tip. This general condition remains unchanged to the tenth day which is as far as the study of sections could be carried with any pretence of general accuracy.

The parenchyma showed some sign of reorganization in one day and there was a considerable apparent difference on the second day, but thereafter there was no appreciable difference from the ordinary conditions.

Bloodvessels were not in evidence in the regenerated tissue until the second day when some were seen which were evidently functional almost to the distal extremity of the new tissue. After the second day a fairly large vessel is uniformly found just beyond the distal end of the nerve cord, and some capillaries were identified quite near the tip in almost all specimens. Many of these were easily observable in the live specimens.

The first sign of extension of the nerve cord consists in a comparatively loose mass of nerve cells or nuclei at the extreme end on the first day. These have some appearance of migration. Little difference appears on the second day although a distinct lengthening of the cord has taken place. The third day shows nearly a half millimeter of regenerated nerve cord but the end shows no very definite sign of rapid growth. After that the condition cannot be distinguished from the ordinary.

The notochord shows some renewal in one day and a slight proliferation on the second after which it appears normal except for a very small area at the end which retains the proliferating character. This is true till about the fifth day when the distal one half to three-fourths of the notochord is markedly different in appearance from the rest. The cells are smaller and more strongly stained, even the cytoplasm taking the hæmatoxylin stain slightly. Careful study of the slide indicates that this distal part of the notochord is in a transitional stage of differentiation and that it has appeared by a simultaneous change in the new cells destined to form the distal notochord. It is interesting to note that whereas on earlier days the notochord had lagged behind the nerve cord it quickly became coterminal with and even extended slightly beyond the nerve cord by the eighth or ninth day. This peculiarity of notochord development is very strongly suggestive as it is coincident with the period of greatest speed in regeneration.

The first sign of muscle development is found on the sixth day when certain cell masses are recognizable as anlagen of the new muscle tissue. These are distinguishable through about one-half the extent of new tissue. On the seventh day a few proximal strands of muscle show fair development. And on the eighth day some are found throughout the proximal third. In nine days a few well developed fibers are distinguishable half way out in the new tissue and on the tenth day it is extremely difficult to identify the border line between the proximal regenerated muscle and the old tissue.

While most of the tissues studied reach the original state of development too soon to show any connection with or influence upon the highest speed of regeneration (Chart II.), it should be remembered that all except the muscle are so generalized in their original condition that they require little change before reaching the original limit of differentiation. On the other hand, the muscle is rather highly specialized and if it be taken as a standard a rather marked correlation is noted. It is just becoming distinguishable at the sixth day when growth has its greatest speed according to this polygon, and it gradually takes on maturity until it is well developed by the ninth day which is evidently somewhat past the time of most rapid extension. A glance at the chart indicates the probability of greatest speed having been shown at about the seventh or eighth day if the temperature had been under control.

Chart II.

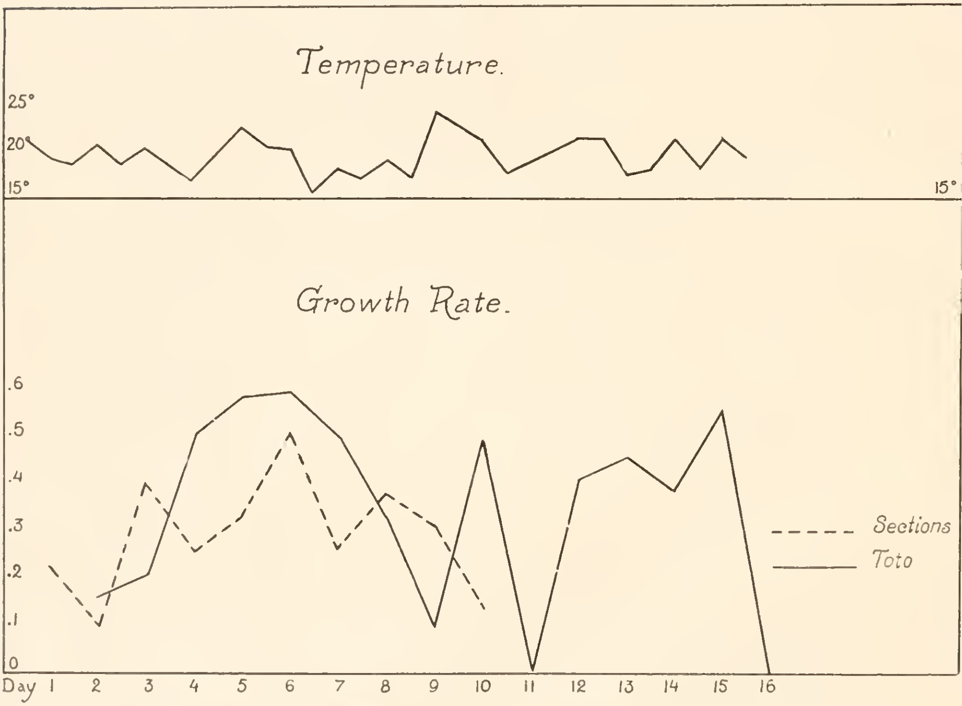


Chart II.—Temperature—1° C. to 1/20 inch rise. Growth rate—0.1 mm. to 5/20 inch rise.

Chart II. is a polygon constructed from micrometer measurements under the compound microscope, of the tissues in toto

TABLE V.

Amblystoma Tadpole

Day.	Integument.	Parenchyma.	Blood Vessels.	Nerve Cord	Notochord.	Muscle.
1	Proliferating.	Slight extension.		Some cell migration.	Slight renewal.	
2	Normal except. distad.	Some proliferation.	Functional almost to tip.	Slight extension.	Some proliferation.	
3	Normal except distad.	Slightly crowded.	Normal beyond nerve cord	Normal, well extended.	Normal except at end.	
4	Normal except distad.	Normal.	Normal beyond nerve cord	Normal.	Normal except at end.	
5	Normal except distad.	Normal.	Normal beyond nerve cord	Normal.	Normal except at end.	
6	Normal except distad.	Normal.	Normal beyond nerve cord	Normal.	Normal except at end.	Traces through one-half regenerated part.
7	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	Normal except at end.	Some proximal fibers well developed.
8	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	One-fourth normal. Rest compact, heavy staining.	Some well formed cells, one-third distad.
9	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	One-fourth normal. Rest compact, heavy staining.	Some fibers one-half distad.
10	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	One-fourth normal. Rest compact, heavy staining.	Some fibers one-half distad.

and after sectioning. The solid line indicates the former and the broken line the latter. Temperature fluctuations are recorded in the upper part. It was very difficult both with the toto examination and the section examination to distinguish the exact plane of cut, and of course, a very slight error in identification of this level would make a marked difference in the polygon. This difficulty rapidly became worse as the regenerated tissue grew older and measurements taken by any method after the tenth day have a purely suggestive value since they have no basis in strict accuracy. A set of measurements was taken with dividers, using a binocular dissecting microscope, but as their results are not essentially different they are not plotted on the chart. Table V. is self explanatory.

Summary.

It seems that the results of these two studies are sufficiently clear to warrant assumptions as follows though further data are very desirable.

1. When adult tissue is removed the resultant regenerative growth reaches its greatest speed just preceding, or at least not later than, the time when the major somatic tissues become typically differentiated.

2. When immature tissue is removed the resultant regenerative growth rate shows no relation of any sort to the differentiation of the more generalized somatic tissues. There is, however, a marked correlation with such a highly specialized tissue as muscle, the differentiation of which is coincident with the period of maximum growth rate. And the peculiar development of the notochord also strongly supports this view.

Of the literature available, only that which was found distinctly useful is listed here. Krecker's list is good and a more general bibliography, especially concerning annelids, can be obtained from his paper.

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ABBREVIATIONS.

R = regenerated tissue.
S = mature segment in old tissue.
RS = newly regenerated segment.
b = bloodvessel.
c = chlorogogue.
e = epidermis.
m = muscle.
n = nerve cord.
nr = nephridium.
pe = peritoneum.
pr = proliferating mass.
s = septum or septal anlage.
ss = seta sac.

EXPLANATION OF PLATE I.

All figures are from camera drawings and partly diagrammatic. All have the same magnification (375 diam.). None are intended to represent cell detail. All show only most conspicuous features of the ventral side in or near the tenth regenerated segment.

FIG. 1. Third day. Septa almost formed. Muscles just distinguishable. Proliferation of epidermis and embryonic cells just inside muscle layer.

FIG. 2. Fourth day. Septa formed. Epidermal proliferation less. Cells massing to form nerve cord inside the muscle layer.

FIG. 3. Fifth day. Nerve cord, bloodvessel and peritoneum formed. No proliferation.

FIG. 4. Ninth Day. Typical ventral localization of cells in nerve cord.

FIG. 5. Old, mature segment. Note larger size, the thin epidermis and thick muscle layer.

FIG. 6. Eighth day. Optical section. Immature nephridium. Mature seta sac and muscles.

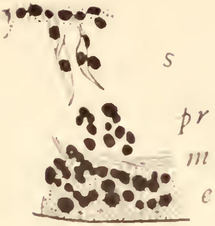

Fig. 1

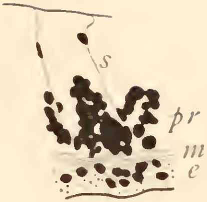

Fig. 2

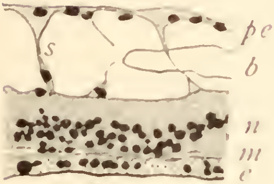

Fig. 3

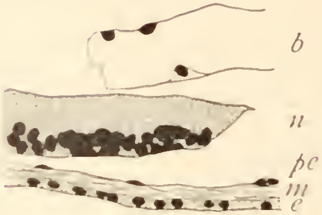

Fig. 4

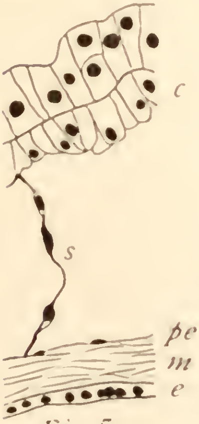

Fig. 5

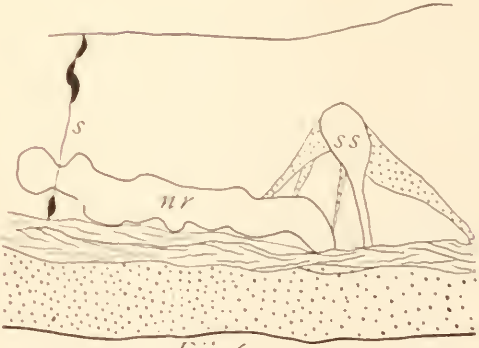

Fig. 6

W. E. ALLEN.

THE SMALLEST POLYODON.

THOMAS BARBOUR.

An article which appeared in a recent BIOLOGICAL BULLETIN by Danforth, entitled a "74 mm. Polyodon"[1] reminded me that some years ago I had seen some small specimens of Polyodon in the study collection of Fishes in the Museum of Comparative Zoölogy. A reëxamination of the material preserved there revealed three examples, the smallest hitherto recorded, as well as others which are among the smallest known. The three important examples are 35 mm., 37 mm. and 65 mm. long. The last individual was somewhat dried and was obviously similar in form and development to the specimen described and figured by Danforth. The smallest has been badly softened and swollen by immersion in weak alcohol. This individual was collected in Arkansas by Mr. G. Stolley and came to the museum before 1860. The other two small specimens as well as three more, which are 80 mm., 85 mm. and 93 mm., came from near St. Louis, Mo., and were collected in September, 1854, by a Dr. Eastman. They were in the private collection of Professor Louis Agassiz which was acquired by Harvard University as the foundation for the Museum of Comparative Zoölogy. These five examples have been kept in strong clear alcohol and are well preserved. The example of 37 mm. then is the important specimen in that it is the smallest known which is reasonably well preserved. Professor C. H. Eigenmann, who has seen this specimen, suggested that it was probably about three months old, in which case the egg laying would take place in July. If this were not really the case it is quite possible that Danforth's individual which was about 3 inches long was not the swift-grown young of the year but a specimen which had lived over one winter. Growth may be very slow or almost arrested during the cold months in the latitude of St. Louis. If, as is quite possible, the 37 mm. young was really much younger than three months, then this rate of growth would seem reasonable enough. Emphasis is laid on this point because great search has been made for the young during the months of spring and early summer. This search has been fruitless, while these inch-long fishes were got in September.

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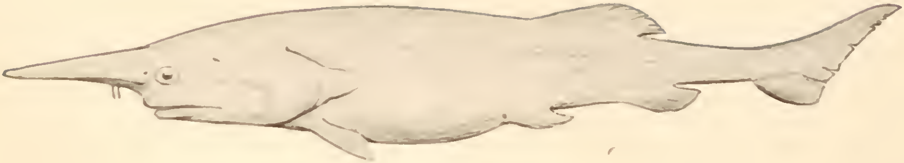
[1] BIOL. BULL., XX., 4, 1911, pp. 201–204.

The drawings which have been made by Mr. E. N. Fischer with special regard to the proportions, show three aspects, dorsal, lateral, and ventral of the 37 mm. example, as well as of one 80 mm. long; two aspects of a fish 130 mm. long and a lateral view of a three-foot adult. These figures serve to show the remarkable features of the young as well as the changes in form which the species undergoes during growth, better than would a lengthy verbal description.

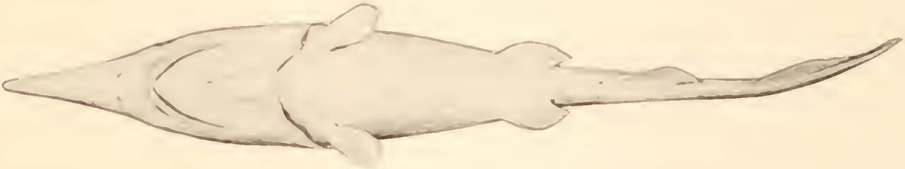
As may be readily seen from the drawings the most interesting characters to be observed in the young are the short sturgeon-like snout which has not yet become in the least spatulate; the extremely heterocercal form of the tail; the relatively long barbels and the less developed fins throughout. Unfortunately the notch near the outer extremity of the caudal fin is not shown n the very young because of the frayed margin of the tail fin. This character is marked in the slightly larger examples and, as is shown in the lateral view of the 80 mm. specimens, the notch marks the point where the cartilaginous fin rays cease to be developed. This character would seem to suggest that seen in the caudal fins of certain sharks where there is a similar notch. The whole general form of this, the smallest known selachostomous ganoid is certainly most strikingly shark-like as well as reminiscent of the adult forms of other ganoid genera.

EXPLANATION OF PLATE I.

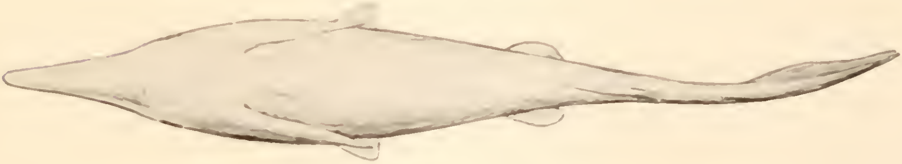
37 mm. Polyodon, × 3. Fig 1*A*, lateral view; Fig. 1*B*, ventral view; Fig 1*C*, dorsal view.



1*A*



1*B*



1*C*

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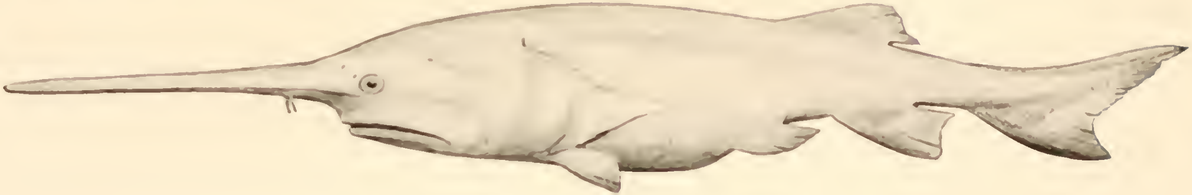
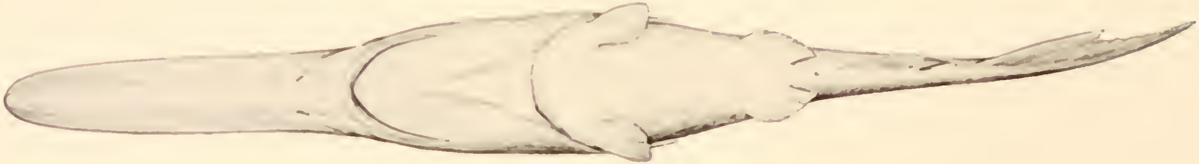
Explanation of Plate II.

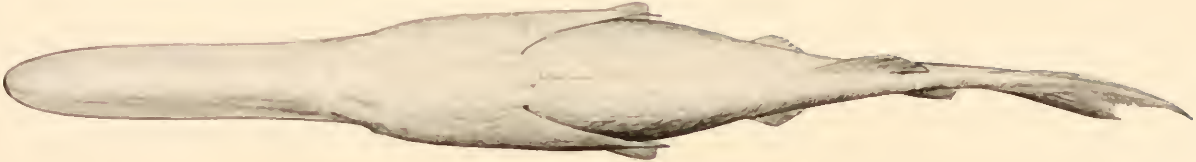
80 mm. Polyodon, × 2. Fig. 2*A*, lateral view; Fig. 2*B*, ventral view; Fig. 2*C*, dorsal view.



2A



2B

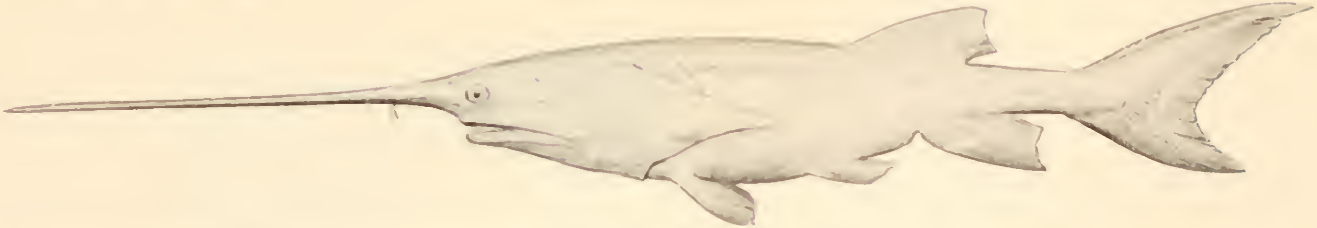


2C

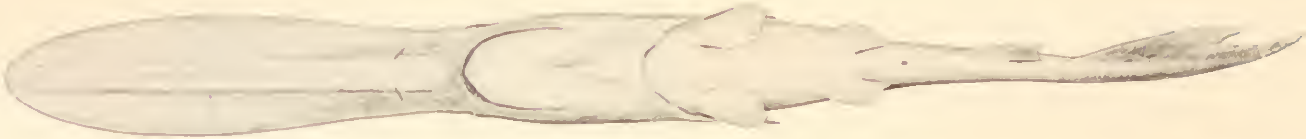
E. N. FISCHER DEL.

EXPLANATION OF PLATE III.

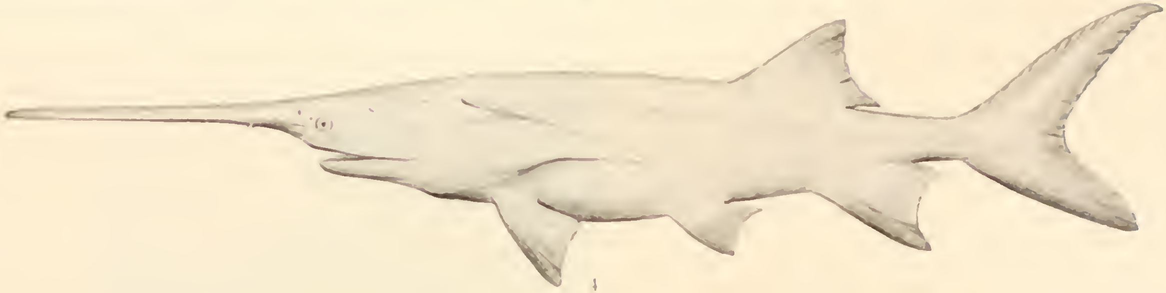
Figs. 3*A* and 3*B*, 130 mm. Polyodon, lateral and ventral views. ×1⅓. Fig. 4, 3 ft. individual from Boston market, 1/6 natural size.



3A



3B



4

E. N. FISCHER DEL.

NOTES ON TWO CROSSES BETWEEN DIFFERENT RACES OF PIGEONS.

T. H. MORGAN.

The present note is intended to record the results of a few crosses with pigeons. Through a series of accidents the experiment came to an untimely end. The breeding carried to the second generation has given too few individuals to warrant any generalizations, but enough to suggest the desirability of more extended work along the same line. As it will take two years to carry the crosses again to the second generation it seems worth while to record the facts so far obtained.

CROSS BETWEEN A WHITE FANTAIL AND "SWALLOW."

A pure white female fantail (Fig. 1) was paired to a male swallow (Fig. 2). One of the chief features of the breed of fantails is the large number of tail feathers; in the bird used here 32 feathers were present. I wished to find out how this

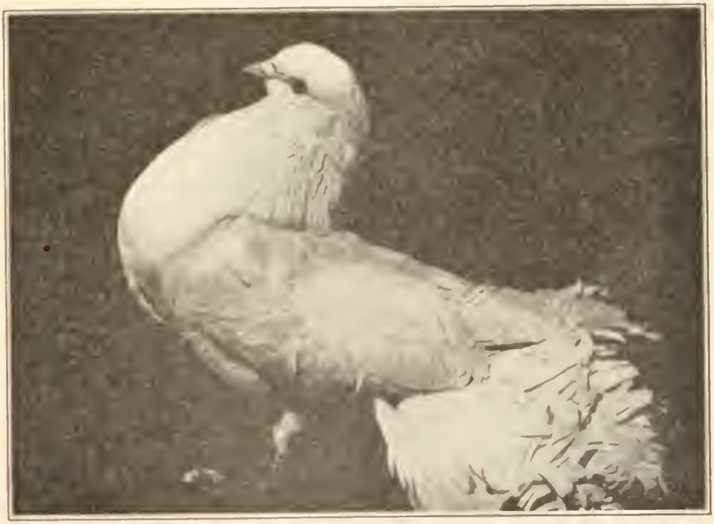


FIG. 1.

character would behave when brought into connection with the normal number (12) of tail feathers possessed by the "swallow."

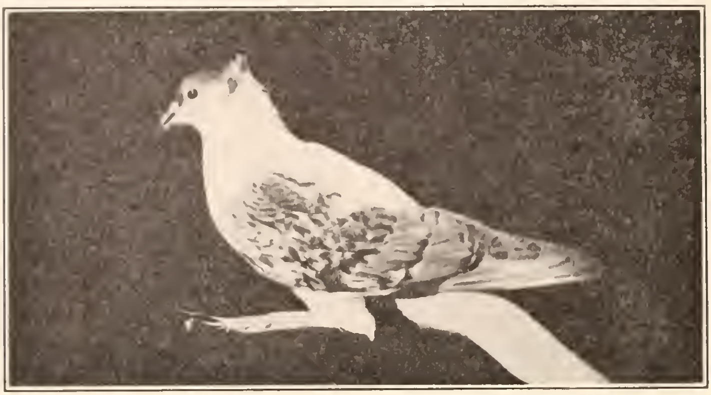


FIG. 2.

The First Generation.—Seven young were reared from this cross. The tails of these hybrids had respectively 17, 12, 13, 15, 14, 13, 13 feathers. The result shows an intermediate condition between the parent types, with a distinct approach to the normal. The tail of the hybrid was not carried in the erect position as-

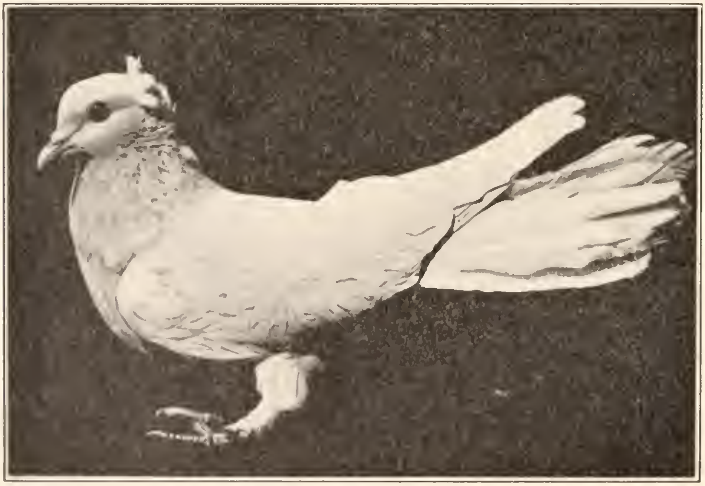


FIG. 3.

sumed by the fantail nor was it flat as in the other type, but formed a broad rather flat wedge like that of the fantail at rest, but not as high. It is shown in Fig. 3.

The F_2 Generation.—Only four birds were reared in this generation. Each had only twelve feathers in its tail. It may appear either that there is a further loss of tail feathers in this generation or that the normal number, twelve, represents the dominant type, and that the number of individuals is too small for the recessive type to reappear. Larger numbers must be obtained to settle this point, for, it seems not improbable that F_2 birds with more feathers than twelve are expected, even although the original number 32 may seldom or never reappear.

Other Characters.—It is not the object of this note to examine in detail the inheritance of other characters of these two breeds, but the following points may be mentioned. The fantail has a plain head (Fig. 1.), *i. e.*, no crest; the swallow has a crest. The F_1 birds all possess a crest. Some of the F_2 birds have a crest, others lack it. One bird that was killed is not recorded for crest.[1]

The swallow has feathers on the tarsus and toes (Fig. 2), the fantail lacks these (Fig. 1). The F_1 birds have feathers on tarsus and toes, but less developed than are those of the father. Two of the F_2 birds lack feathers on the tarsus and toes, one bird resembles the F_1 hybrid, and one has well feathered tarsus and toes. In the last case the feathers are as long, and as well developed, as in the swallow.

The fantail was pure white, the swallow has a dark top to the head, dark wings, and the feathers of the feet are light bluish. The wings are grayish blue and brown with two brown bars (Fig. 2). The tail, back, neck and ventral surface are white. The F_1 birds are pure white. Two of these birds showed two or three slate-colored feathers near the outer edge of the wings, generally to be seen only by brushing back the white feathers or when the wing is extended. Two of the F_2 birds are pure white, two were splotched, one with red, and one with brown. Only a small number of these colored feathers were present, but they were scattered unequally over the body and showed no tendency to be grouped on the wings and head alone.

[1] Owing to injury by rats the head was too much eaten to give certain records for the crest.

Summary.—The F_2 generation was intermediate in number of tail feathers, feathers on legs, and color, although in regard to the last point the birds were practically like the white parent, a few feathers alone placing them in the intermediate class. The crest was dominant. The F_2 generation showed segregation practically complete for the feathers on the legs, it showed segregation of white color to some extent, the recessive type appearing only to a very limited degree. The number of tail feathers showed a return to the normal, but the number of individuals is too small to lay much emphasis on the point. Yet it is significant that in all other directions the F_2 birds sufficed to show the influence of both parents.

Cross Between a Turbit and a Starling.

The F_1 hybrids of those birds were given to me by Mr. E. B. Southwick, who kept both parent stocks. The turbits (Fig. 4) were not very high-bred strains, but showed the main characteristics of their class. Their color is red or blue. The head is uniformly colored and has a crest: the wing bars are dark; along the middle line of the breast there is a series of reversed feathers (Fig. 4). The starling (Fig. 5) was a black bird with a white top to the head and white wing bars. On the breast there is a large light crescent with a metallic tinge. There are no reversed feathers on the breast.

The two hybrids (F_1) were red birds (Fig. 6). The crest was well developed. The top of the head was white (like that of the starling), but in one case not so sharply delimited. The wing bars were dark.

From these two hybrids eight, F_2 birds were reared. Five have crest and two lack the crest, five are red and three blue, all but one have darker bars on wing. This one has a dark bar on most of the feathers of the wings but a few feathers have white bars.

Summary.—The most interesting result of this combination is the failure of the reversed feathers on the breast to reappear in the second generation. The result is like that of the number of tail feathers in the fantail-swallow cross, but here also the numbers are too small to make any conclusions possible, however significant the absence of reversed feathers may appear. The *white* top of the head and the creast appear to be dominant; while the *white* wing bars are recessive.

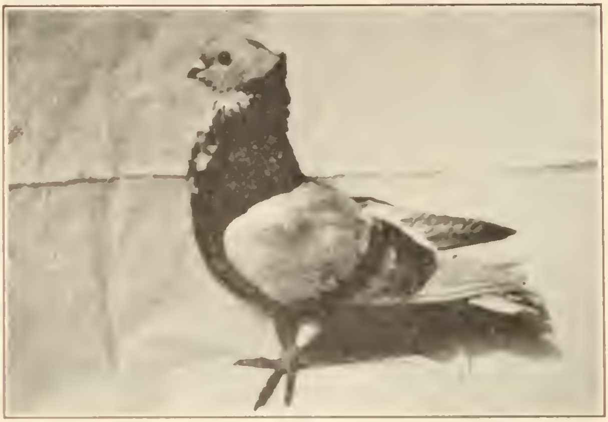


FIG. 4

CONCLUSIONS.

As stated above the number of individuals reared is too small to warrant any extensive generalizations, but it is apparent that while certain characters show evidence of mono-hybrid inheritance, three characters do not furnish as good evidence. These three characters are the number of tail feathers in the fantail, the color pattern of the swallow, and the reversed breast-feathers of the turbit. It is probable that each of these characters has not been formed by a single step, but by a series of steps.[1] If this be granted it follows that the conditions present in the two races respectively can not be represented by a pair of Mendelian characters. There are then two interpretations; first, if the characters Mendelize there must be more than two factors involved; second the characters in question may come under some other kind of inheritance in which simple Mendelian segregation does not occur, or possibly the results may be partly Mendelian, partly not. A large number of F_2 offspring will be necessary before these points can be settled, but enough has been found, I think, to show at least in cases like these when a type

[1] See Morgan, T. H., "Experimental Zoölogy," page 171, New York, 1907.

has been formed either under domestication, or in Nature, not by one mutational step alone, but by a series of successive steps along the same line, that the crossing with the original type or with a related type does not give Mendelian inheritance for one pair of characters. What we should expect under such circumstances we do not know as yet for we are now dealing with a

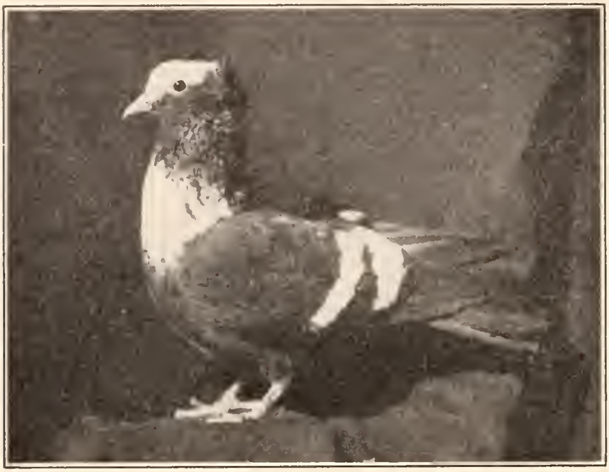


FIG. 5.

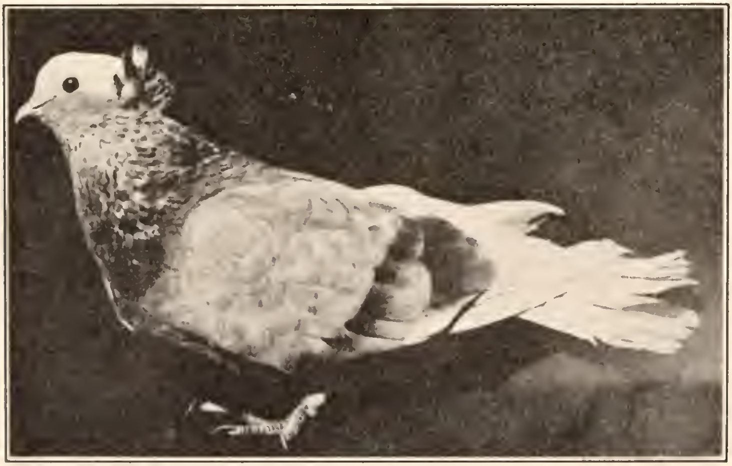


FIG. 6.

series of progressive or orthogenetic variations and not with a retrogressive mutation.

There is another result that seems to me to be especially significant. The white on the top of the head of the starling is dominant to the plain (colored) head of the turbit, but the white wing bars of the starling are recessive to the dark wing bars of the turbit. Unless these are two different kinds of white, one dominant (inhibitor of color) and the other recessive (absence of color) the result is difficult to explain, unless as I have suggested[1] the segregation in mosaic types is not germinal but ontogenetic.

[1] "The Influence of Heredity and of Environment in Determining the Coat Colors in Mice," *Annals New York Acad. Science*, XXI., July, 1911.

THE ŒNOCYTES OF PLATYPHYLAX DESIGNATUS WALKER.

WILSON P. GEE.

Introduction.

The œnocytes as unique cells of the body of all of the orders of insects which have been investigated, except the Aptera, have received much attention within the last couple of decades, but with relatively little more than mere conjecture as to their definite function. With the idea of throwing added light on the nature of the activities of these cells, the investigations here discussed were made on the larvæ, pupæ, and adults of *Platyphylax designatus*, a caddis-fly, which occurs quite abundantly in fresh-water springs in the vicinity of Madison, Wis.

The results here presented were derived from a study of the structure of the œnocytes in several periods of the life-history of this insect, and by injection experiments. Several stains were tried, Heidenhain's iron-hæmatoxylin proving the best for detailed work. Delafield's hæmatoxylin with orange G or eosin were the stains used in determining the location and number of the œnocytes. The cytoplasm of the œnocytes takes up eosin very readily, as it does also orange G. The substances used in injections were methylene blue as an intra-vitam stain and sulphindigotate of sodium to test the excretory nature of the cells.

The papers of Perez (1903), Rössig (1904), and Weissenberg (1907), presenting a rather complete review of the work done on œnocytes, it will here be necessary only to briefly mention some of the chief contributors to the subject.

According to Rössig (1904), the first to observe the œnocytes as special organs was M. Fabre (1856).

Landois (1865) distinguished them from the fat-body, and gave to them the name "Respirationszellen" because of the relation he observed them to bear to the tracheæ.

Haberlandt (1872) observed them in the silk-worm, where they were described by him as an organ of unknown function.

Graber (1873) separated the œnocytes sharply from the pericardial cells, and called them "eingesprengte Zellen." He said regarding their function: "Ohne Zweifel haben wir es hier mit eigenartigen einzelligen Drüsen zu thun, über deren secret allerdings nicht das Geringste bekannt ist."

The name "Oenocythen" was given to these cells by Wielowiejski (1886) on account of their resemblance in color, in the larva of *Chironomus*, to white wine. They were observed by him to be metamerically repeated under the hypodermis of the abdominal segments and arranged in small groups surrounding or near to the stigmata. This was the most extensive work on the œnocytes up to his time; for Wielowiejski was successful in identifying them in the Diptera, Coleoptera, Hymenoptera and Lepidoptera. He includes the œnocytes among the elements of the blood tissue and says regarding these: ". . . dass sie alle von dem sie umgebenden Medium gewisse Stoffe aufnehmen, zeitweise aufspeichern resp. verarbeiten und irgend welche Umsatzprodukte an dasselbe zurückgeben und dadurch auf die in den Hauptgeweben des Organismus vor sich gehenden Assimilations- und Desassimilationsprocesse einen Einfluss ausüben."

Kowalevsky (1887) attributed to the œnocytes a glandular function.

Verson (1891–2) in his work on the silk-worm gives them the name "cellule glandulari hypostigmatische," and finds their location exclusively under and behind the stigmata of the abdominal segments. As evidenced by the number of vacuoles in their cytoplasm during the larval stages, he attributes to them a glandular function. At the time of the spinning of the cocoon, two or three days before pupation, a second œnocyte generation appears in broad layers on the ventral part of the third, fourth, and the fifth abdominal segments. These attain a size of 60μ in the six-day pupa, and begin at this time to divide by amitosis. The larval œnocytes persist to the end of the life of the individual, reaching as much as 136μ in size.

Graber (1891) places the œnocytes with the blood tissue and gives their origin as ectodermal. He, however, considers them as being metamorphosed into the fat-body, and also as giving rise to the blood corpuscles.

Wheeler (1892) in an extensive paper on the blood tissue of insects deals with the œnocytes at considerable length. He arrives at the following conclusions:

1. "The œnocytes are derived by delamination from the ectoderm just caudad to the tracheal involutions They are also metameric organs.

2. "They are limited to the eight trachigerous abdominal segments.

3. "They appear to be restricted to the Pterygota, in all the members of which group they probably occur.

4. "They give rise neither to the fat-body nor to the blood but represent organs *sui generis*.

5. "After their differentiation from the primitive ectoderm, they never divide, but gradually increase in size."

With regard to their function, the only suggestion that Wheeler makes is in his discussion of their vacuolate structure in certain of the larvæ of the Trichoptera. Here he says: "One is reminded of certain gland cells which store up vacuoles of a specific substance in their cytoplasm, preparatory to secretion."

Heymons (1895) in the embryogenesis of *Forficula*, points out that the œnocytes are found in the eleventh abdominal segment as well as in the others. The eleventh segment bears no stigma, and thus they cannot bear any relation to the stigma as such.

Pantel (1898) classes the œnocytes as an organ because of their metameric repetition, and treats to a slight extent of their cytological changes in different periods of the insect's life. He hesitates, however, to commit himself as to their function, but rather inclines toward the excretory phases of their probable activities.

Berlese (1899), in his work on the ants, *Tapinoma erraticum* and *Pheidole pallidula*, finds that, in the beginning of metamorphosis, the larval œnocytes are multiplied, give up their fixed position, and, becoming amœboid, are scattered about among the fat-bodies. In his work on *Polistes gallica* and on the honey-bee, he finds a persistence of the œnocytes, with no special second generation. Their function he considers as excretory, serving during the periods of moulting and pupation, when the Malpighian tubes are functionless.

Anglas (1900) describes the œnocytes as more or less amœboid, and with no special distribution in the Hymenoptera, except their existence in the abdomen alone. Except for a relative increase in volume, they remain throughout the life-cycle, in both distribution and structure, very similar to what they are in the early larval stages. He suggests that they perhaps secrete about themselves ferments, and considers them as glandular cells originating from the hypodermis and functioning as organs of internal secretion perhaps for dissociative purposes. The products of their secretion may serve for the general nutrition of the tissues of the body or even for the cytolysis of those larval cells destined to disappear.

Koschevnikov (1900), in his work on the fat-body and œnocytes of the honey-bee, finds that most of the large œnocytes of the larva disintegrate in the pupal stage; some of them continuing, however, into the beginning of the imaginal stage. In a young pupa, a second œnocyte generation, to which he gives the name "Imaginalœnocyten," arises from the hypodermis, and these œnocytes are later distributed among the fat-bodies. These are only one fifth as large as the larval œnocytes and are formed only in the pupal stage. He observed inclusions in some of the cells, and on this basis came to the conclusion that they are to be considered as excretory organs, without "Ausführungsgange," which serve as storage places for excess products of excretion.

Vaney (1902) says: "Chez la *Simulia*, le *Culex*, le *Chironomus*, durant la période nymphale je ne vois aucun changement dans ces cellules." However, he observes in *Simulium* that the number of œnocytes appears to have increased at the end of pupation. He concludes: "Chez les *Dipteres* les œnocytes ne subissent aucune histolyse durant la nymphose."

Perez (1903) states relative to the discovery of Koschevnikov, concerning the origin of a second œnocyte generation, that this opinion seems to be erroneous.

Rössig (1904) finds that, in the Cynipidæ, the œnocytes are limited to the abdomen. The larval œnocytes reach a length as great as 150μ and attain a proportionate length one fifth that of the body. These larval œnocytes degenerate in the pupal stage. The imaginal œnocytes originate at about the same time through mitotic division of the hypodermal cells on the ventral side of the abdominal segments. They are described as very much smaller than the larval œnocytes, never equalling them in size.

Weissenberg (1907) finds in a well grown larva of *Torymus nigricornis*, over one hundred œnocytes, presenting no definite arrangement, and scattered about among the fat-bodies. They are in no way limited to the abdomen, nor do they necessarily have the definite tracheal attachments observed by Wielowiejski and others. The principal degeneration of the larval œnocytes occurs in the stage which he calls the "gelben Puppe." The larvæ shortly before pupation contain the imaginal œnocytes in the form of special œnocyte imaginal plates, which are laid in the niches of the dorsal hypodermis in the fifth to eleventh abdominal segments.

With regard to the function of these cells he says: "Schon oben habe ich mich gegen die Auffassung der Zelleinschlüsse als Excrete ausgesprochen, möchte aber hier nicht das für Urate characterische Bild der braunen, radiär gestreiften, runden Krystallkörper darbieten, sondern an den frischen Zellen homogen und farblos erscheinen.'

Original Observations.

In the larvæ of *Platyphylax designatus*, the œnocytes are limited to the abdomen. A careful search through serial sections of the thorax of several individuals in different larval stages showed no traces of œnocytes in this portion of the body. They are in no way limited, however, to certain abdominal segments but occur in all, the larger number being present in segments two to seven. The definite tracheal attachments observed by many of the workers on this subject have not been seen in this insect. This observation agrees essentially with that of Weissenberg (1907) in *Torymus nigricornis:* "Von einer Befestigung an Tracheencapillaren, habe ich nichts bemerkt."

The œnocytes occur both dorsally and ventrally, by far the greater number occupying the latter position. The larger œnocytes, so conspicuous in the early larval stages, occur ventrally in pairs, one on each side of every abdominal segment. The smaller œnocytes of the earlier, as well as the later larval stages, are found principally in groups of from two to five, the larger groups appearing more ventrad. In several specimens they were observed near to or even grouped around a tracheal tube to which they, however had no connection. The location of all of the œnocytes is distinctly pleural.

The distribution of the œnocytes does not change essentially with the increase in size of the larvæ, but there is a less marked tendency for them to occur in groups. With the increase in size of the fat-body more of them become partially or completely surrounded by this structure. These changes are more marked in the late larval and in the pupal stages, but we frequently find in these periods, groups of as many as five œnocytes, and this tendency to grouping is observed even in the imaginal stage.

A careful count of the œnocytes of a larva eight millimeters in length totals about one hundred and twenty-five, these being approximately equally proportioned between the right and left sides of the body. From other estimates it is found that this number is about typical for *Platyphylax designatus*. There seems to be no wide fluctuation in numbers in different periods of the life-history, though the imaginal stages appear to show a decrease in number over the larval, due perhaps to disintegration. The conclusion that the number is constant for all the larvæ of the same stage, is, I believe, hardly justifiable, but there is no wide variation in this regard. In no case, have I seen evidences of division either by mitosis or amitosis in any of the stages studied, nor an increase in number in later larval, pupal or imaginal stages over that in the earlier larval periods.

While the œnocytes appear practically constant in number throughout the stages examined, there is to be noted a very marked increase in their size as the larva advances in growth. The following table represents the size of an average œnocyte derived from measurements of several of these cells in the stages indicated.

The largest size of the œnocytes is reached in the pupal stage; the greatest length of the nucleus proportionate to that of the

Length of Larva.	Length of Cell.	Length of Nucleus.
4.5 mm.	18.4μ	10.4μ
6 mm.	24 μ	12 μ
12 mm.	40 μ	20.6μ
22 mm.	77 μ	43.5μ
pupa	103.1μ	63.5μ
early imago	94.3μ	62.9μ

cell is found in the imaginal stage. There is to be noted, however, a decrease in the total length of the œnocytes of this latter stage, this being due, beyond doubt, to degeneration, manifest signs of which are present.

There are two sizes of œnocytes in the earlier larval stages. One of the larger type, from a larva six millimeters in length, is shown in the accompanying plate (Fig. 3). As already noted, these large œnocytes are present in each of the abdominal segments, ventral in position, one occurring on each side. These are many times larger than the œnocytes of the smaller type, and are pressed rather closely against the hypodermis. In the earlier stages of larval life, the greater comparative size makes these œnocytes very conspicuous, while in the older larva this is not so marked; in fact the smaller œnocytes in the oldest larvæ attain a size equal to or even greater than the formerly larger ones. Careful examination of serial sections of these larger œnocytes showed no traces of a duct leading from them.

The smaller œnocytes are more numerous than the larger. In the early larval stages, they are, as noted above, much smaller than the other type, attaining, in the old larvæ, an equal or greater size. Their distribution in the body is, however, very much more irregular.

In the earlier larval periods the œnocytes appear much more rounded in form than in the earlier stages. The nucleus shows the same tendency. The cytoplasm presents a finely granular, homogeneous appearance, and takes up rather evenly the orange G and eosin. In these stages, however, the cytoplasm appears rather more dense than in the succeeding periods.

Wheeler says with regard to the œnocytes of an unidentified phryganeid larva on which he worked that the nuclei contain no *nucleole*. In a 4.5 mm. stage of *Platyphylax designatus*, stained with safranin, a nucleole is seen to stand out very clearly (Fig. 1).

This apparently disappears in later stages. The bulk of the chromatin appears in small, rounded granules connected to form a skein. One easily observes several larger masses of chromatin standing out rather conspicuously in the nuclei of the œnocytes of all the larval stages. The nuclear membrane appears sharply defined in all of the stages except the imaginal.

Besides a relative increase in the size of the cell, there appears little change in the character of the œnocytes up to the last larval stage. At this period the nucleus becomes proportionately much larger and more irregular in outline. The cytoplasm shows, between the nuclear membrane and cell boundary, an inner and an outer zone or layer. The more central of these two layers shows the cytoplasm to contain more and larger vacuoles, and to have a denser appearance; the outer, smaller zone, on the contrary, contains fewer and smaller vacuoles. None of these vacuoles are clear, but one may distinguish in the larger ones, by careful focusing, many very small granules, which would seem to indicate the presence of more than a homogeneous content. These vacuoles have been observed by Verson (1891–2), Wheeler (1892), and others, all of whom interpret them as indicative of secretory activity. Wheeler (1892) says: "These vacuoles are but slightly refractive, and are not fat globules. This condition of the œnocytes was found in a number of larvæ, and I believe, represents a normal physiological state. One is reminded of certain gland cells which store up vacuoles of a specific substance in their cytoplasm, preparatory to secretion."

In the pupal stages, one finds that the nucleus has a more irregular outline, and that the cytoplasm contains comparatively few vacuoles. It is in this stage that the œnocytes attain their largest growth. This fact coupled with the appearances of activity in the late larval stages may perhaps be taken as indications of a greater secretory activity of these cells during these two periods.

In the imago, just after emergence, the œnocytes show marked signs of degeneration. The boundary of the cells has become indistinct and frayed, and the outer portion of the cytoplasm is stained rather deeply with the hæmatoxylin (Fig. 7). At this stage, the nuclear membrane is not distinguishable, and but few vacuoles are to be noted in the cytoplasm. The chromatin has gathered into irregular masses along the sides of the adjacent cytoplasm (position of former nuclear membrane) and the linin appears in masses which have separated from the chromatin material. In an imago two or three days after emergence, but which had been kept enclosed in a small dish, the degeneration of the nucleus is seen to have proceeded further. The cell does not, however, present an essentially different appearance to that shown in the imago two or three hours after emergence. This third-day imago was the latest stage of which material was available. It would be interesting to determine from older imagoes whether the œnocytes entirely degenerate and are taken up for the purpose of general nutrition of the tissues, thus affording a reserve food supply in addition to their secretory function. This latter view would, however, be hard to understand considering the short life of these insects, and also the fact that egg-laying goes on shortly after emergence from the pupal case.

Various functions have been attributed to the œnocytes. Landois (1865) gave to them the name "Respirationszellen" and assigned to them a place in the respiratory system. Graber (1873), in his early work, speaks of them as unicellular glands, the secretion of which was of unknown function; later, however, he holds that they are metamorphosed into the fat-body, and give rise to blood corpuscles. Berlese (1899) considers the œnocytes as excretory in function, as does also Koschevnikov (1900).

Anglas (1900) says: "Ce fait permet d'affirmer qu'ils sécrètent autour d'eux des ferments. Aussi les considérons-nous comme des cellules glandularies (nées de l'hypoderme) et jouant le rôle de glandes à sécrètion interne, mais de glandes dissociées." Verson (1891–2) considers them as secretory in nature as do also Rössig (1904) and Weissenberg (1907).

Holmgren (1900) in *Apion flavipes*, experimenting by injecting small quantities of powdered methylene blue and alizarin-cyanin into the body cavity, came to the conclusion that they were excretory in function. He found that the œnocytes took up the color in much the same manner as the Malpighian tubes, the nucleus of the œnocyte showing signs of staining somewhat earlier than the nuclei of the latter organs. Three hours after injection, he found that the methylene blue had passed into the lumen of the Malpighian tubes in sufficient quantities to color them; the œnocytes at the end of an hour and a half had lost their color.

Following out this line of investigation, a dozen or more specimens of *Platyphylax designatus* were injected with an aqueous solution of methylene blue and the parts dissected and removed to a glycerine mount. It was found that immediately after injection the œnocytes and the spinning-glands, so prominent in this form, had both taken up the stain—the œnocytes to a much less marked degree than the latter. In only one instance, was it found that the Malpighian tubes had taken up the methylene blue, and in this case the color was seen in but a single tube. The œnocytes took up the stain more in the nucleus than in the cytoplasm, the latter, however, being distinctly tinged. The larvæ killed a half hour after injection showed the œnocytes more deeply stained, but no coloration was observed in the Malpighian tubes. Larvæ killed one hour after injection showed that the Malpighian tubes had begun to take up the blue color, but that the œnocytes and spinning-glands at this time were becoming less intense in their coloration. These results would lead one to rather different conclusions than those reached by Holmgren. It would be inferred from the similarity of the reaction of the œnocytes and spinning-glands as detailed above, that the former are secretory though of a different nature and intensity to that of the spinning-glands.

This view is further strengthened by injection experiments with sulphindigotate of sodium. Fürth (1903) says: "Die exkretorische Bedeutung der Malpighi'schen Schläuche offenbart sich auch in unzweideutiger Weise durch ihr Vermögen die Ausscheidung von Farbstoffen zu bewerkstelligen." Schindler (1878) found by injection of a solution of sulphindigotate of sodium into the body cavity of *Gryllotalpa* that the Malpighian tubes excreted the substance within twenty-four hours.

An aqueous solution of sulphindigotate of sodium was injected into the abdomen of a dozen or more larvæ of *Platyphylax designatus* 15–20 mm. in length, the insertion of the hypodermic syringe being ventral and thoracic, but the solution extending thoroughly into all the space between the hypodermis and the digestive tract. Specimens killed within an hour after injection and mounted as in the former experiment, showed a slight accumulation of the solution in the lumen of the Malpighian tubes. This was even more marked two and a half hours and four hours after injection, a diffuse blue appearance being then very apparent throughout the cells of most of the tubes. The œnocytes, however, showed no signs of coloration at any of the periods observed nor any evidences of excreting the substance injected.

Just what the function of these peculiar cells may be it is difficult to say. In no case have I observed in the cytoplasm of any of the œnocytes what could be interpreted as accumulations of solid products of excretion. Their behavior toward methylene blue, and sulphindigotate of sodium would seem to show that they are secretory rather than excretory in function. The nature of their secretion is difficult and practically impossible to determine. Measurements show their greatest size in *Platyphylax designatus* to be in the pupal stage, and evidences from a purely structural basis seem to point towards the greatest functional activity during the late larval and the pupal stages. Their intimate relation to the fat-bodies seems vastly more significant than the occasional grouping about a tracheal tube. Their relative increase in size, while of course proportionate to the increase in size of the body, seems to bear a direct relation to the size and development of the fat-bodies. It is also found that their identity is retained throughout the period of metamorphosis. Can it be that their function is the secretion of a substance or enzyme which is of aid to the fat-body in its constructive work or is it that their function is, as Anglas (1900) suggests, that of internal glands of a dissociative nature, their secretion serving to aid in the elaboration of products for general nutrition of the tissues or for the disintegration of larval cells destined to disappear? These are very pertinent questions, but ones to which a definite answer cannot as yet be given. It seems safe to conclude that they are internal, ductless, secretory glands, whose function may, in the light of recent progress in the field of internal secretion, be for a long time a matter of conjecture.

These investigations were taken up at the suggestion of Prof.

W. S. Marshall, to whom I am indebted for many suggestions in the direction of the work.

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EXPLANATION OF PLATE.

All figures drawn with a camera-lucida. × 630.

FIG. 1. Œnocyte from a 4.5 mm. larva.

FIG. 2. One of the smaller œnocytes from a 6 mm. larva.

FIG. 3. One of the larger œnocytes from a 6 mm. larva.

FIG. 4. Œnocyte from a 12 mm. larva.

FIG. 5. Œnocyte of late larval period showing vacuoles in cytoplasm.

FIG. 6. Œnocyte of pupal stage.

FIG. 7. Œnocyte from newly emerged imago, showing signs of degeneration.

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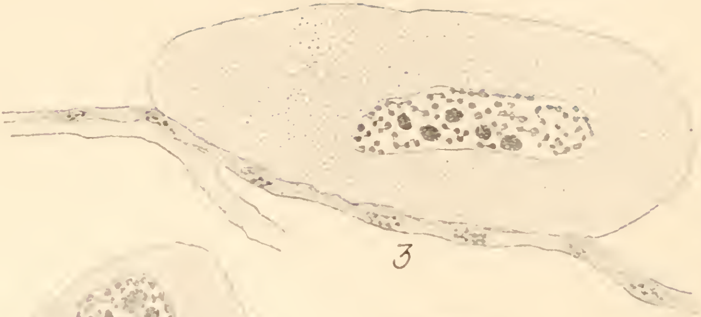

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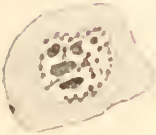

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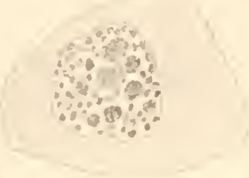

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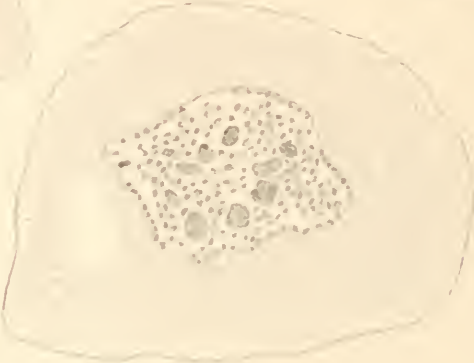

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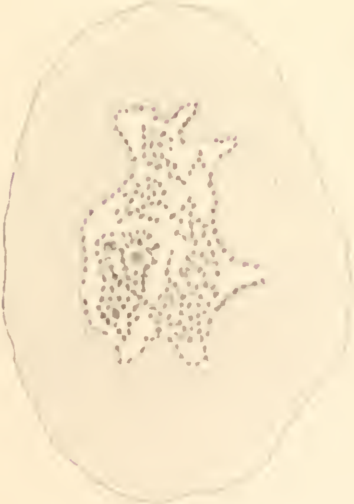

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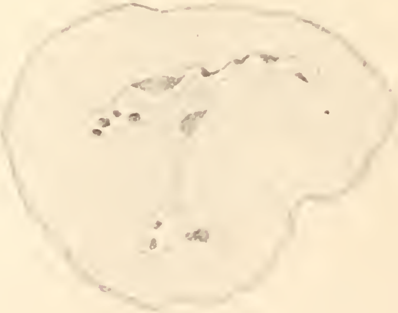

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WILSON P. GEE.

THE MATING HABITS OF FOUR SPECIES OF THE BRACHYURA.

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1. INTRODUCTION.

In the course of a study of the general activity of the crayfish which has occupied my attention for the past three years, I became interested in the mating habits of Arthropoda.

During the summer of 1910, while acting as a temporary assistant in the Woods Hole laboratory of the Bureau of Fisheries, I had the opportunity to observe the mating habits of four species of marine crabs, and to experiment with them to determine if possible their manner of sex discrimination.

In this paper I shall briefly describe the results obtained and consider similar work done by various investigators on the other Arthropoda.

Acknowledgment is gratefully made to the Hon. Geo. M. Bowers, U. S. Commissioner of Fisheries, for the opportunity to work in the Woods Hole laboratory; and to Professor J. P. Porter of Clark University for reading and criticising this paper.

2. OBSERVATIONS AND EXPERIMENTS.

The species observed were: *Callinectes hastatus*, the blue crab; *Cancer irroratus*, the common rock crab; *Carcinus mænas*, the green crab; *Platyonychus ocellatus*, the lady crab.

A description of the differences between the male and the female; the virgin female; the ovigerous female and the ovigerous female about to lay, will apply in general for all of the species.

In the male the abdomen is narrow and the intromittent organs or verges are the first pair of abdominal appendages. The paired penes are formed by the everted posterior parts of the efferent ducts. The penes transmit spermatophores down the grooves of the verges into the seminal receptacles of the female.

In the virgin female the abdomen is much like that of the male, except that the telson is narrower. Hay (15) discovered the presence of a pair of hooks which project from the body and fit into a pocket on either side of the abdomen, holding the abdomen tightly flexed against the ventral surface of the carapace. The swimmerets are very small in the virgin female. Crabs moult several times during the summer, according to Herrick (16).

At the moulting the broad abdomen of the ovigerous female is liberated from its narrow quarters in the old virginal shell, and the female is ready for impregnation. The abdomen of the ovigerous female is held flexed only by muscular contraction.

A few days after impregnation, the female may be seen to have two spermatophore plugs projecting slightly from the vaginæ and effectually closing the entrance. These plugs are probably secreted by the glands of the male. Andrews has found that the spermatophores of the crayfish are held in the annulus ventralis of the female by plugs secreted by the glands of the male (2). It is not known by me just when the plugs are forced out from the vaginæ of the female crab, but probably they are retained until just before the laying. This would be the case if the crabs are to be classed at all with the crayfish in which fertilization is external, and in which the annulus ventralis acts as a *temporary* sperm receptacle.

SPECIMENS STUDIED.

		Females.		
	Males.	Virgin.	Ovigerous.	With Eggs.
Blue crabs	3	3		
Rock crabs	3	2	7	1
Green crabs	2	2	2	
Lady crabs	2	2	2	

In the above table the virgin females are those that were brought into the laboratory and moulted there.

Distinction between the ovigerous females showing spermatophore plugs and those not showing them will be made as the experiments are discussed.

The crabs were kept in a sea water aquarium 2 × 2½ × 4 feet. In the case of the blue crabs, observations and experiments were made only on those confined in this tank. The other species were smaller and individuals were from time to time removed to circular bell jars, 9 in. high and 9 in. in diameter, with about 6 in. of sea water in them.

Blue Crabs.—The female about to moult is seized by a male and carried about by him for a day or two until she is ready to shed her shell. During the pre-moulting period the female is in a normal upright position, being held by the second, third and fourth pair of ambulatory appendages of the male. It is stated by Hay (15) that the male appears able to distinguish the female which is about to moult. I was not able to test this in the blue crabs, but from the observations on the behavior of the rock crabs, green crabs and lady crabs, I am inclined to question the statement and to believe that the male is not able to distinguish the female about to moult except from the fact that she is less resistant. I have noted this sluggishness and apparent weakness in the crayfishes for several days before moulting (7).

When the female is in the process of moulting, she is left for a time by the male. Then he approaches her and turns her over so that her head is beneath his abdomen. I was unable to see the first approaches of the male to the soft-shelled *ovigerous* female, but separated two pairs many times and watched the readjustments.

When the female has been turned so that her ventral surface is apposed to that of the male, and her head is situated beneath his abdomen, both remain quiet for several minutes unless disturbed.

After a period of from thirty seconds to ten minutes, the male taps the female lightly with his first and second ambulatory appendages and turns her slowly around underneath him. The male is not, however, the only active party. No sooner has the female been aroused from her semi-hypnotic state by the movement and tactile stimuli of the male, then she begins to use her ambulatory appendages in turning herself. In order to determine if an external stimulus would cause an early adjustment, I tapped lightly on the edge of the thoracic region of the female, by means of the tip of a small seeker. This was tried on three successive occasions and in each case the female began to move herself into the copulating position by walking around the legs of the male, placing her legs between and against his. In one of these cases, the female moved so rapidly that the male may have thought she was attempting to escape, for he beat upon her carapace in the attempt to quiet her.

It is extremely interesting to see such activity on the part of the female crab, when one has as a contrast the passivity of the female crayfish.

When the female crab has turned about two thirds of the way around, adjustment of the abdomens must be made. As above stated, the abdomen of the ovigerous female is not bound by hooks to the body and is held flexed by muscular contraction. During the preliminary maneuvers, the abdomen of the female is held slightly extended, and the male must push this partially extended obstacle out still farther and interpose his own telson between it and the body of the female. In the case of the blue crabs this is not difficult. The verges of the male are inserted in the seminal receptacles of the female, and copulation takes place. The male carries the female about, grasping her with the tips of his ambulatory appendages, and holding her for hours, sometimes days. While the act of copulation is going on, the female is exceedingly quiet. The male is very pugnacious. Bachelor males were placed in the vicinity of a mated pair of blue crabs and were beaten away by the vigorous defense of the male in copula.

The two male blue crabs under observation were tested as to their recognition of females, hard and soft, of the three other species. In no case did a male attempt to copulate with an alien female.

Rock Crabs, Green Crabs and Lady Crabs.—These species are all smaller than the blue crabs, and are not materially different in appearance from each other. In the case of the blue crabs, I did not succeed in getting a female with spermatophore plugs. In the case of the three species under present consideration, I did secure several ovigerous females that had been fertilized before they were brought into the laboratory, and also secured a rock crab with eggs.

The method of capture of the female by the male is the same in these crabs as it is in the blue crabs. The ability of the blue crabs to distinguish by sight, however, seems to be greater. In handling the members of the different species, one is struck immediately by the remarkable visual powers of the blue crab. He is alert to moving objects and knows when a body comes within striking distance.

Males of all three species were tested by removing the soft females and replacing them by hard males. They attempted in all cases to copulate with the hard males. Then in the case of the rock crab, two males were tested by placing with them, hard, ovigerous females, in which the spermatophore plugs were not protruding. In these cases, the males succeeded in inserting the verges and actual copulation took place.

In the case of the males with whom were placed other males, the approach of the recently copulating male to the stranger male was made in a similar manner to his approach to the lost partner. When the stranger resisted violently, the other male attempted to beat him into passivity just as he successfully did with the females, hard and soft. Just as I have previously held in the case of the crayfish, I now hold that the ability of the male crab to distinguish the sexes is really a matter of inability to hold and copulate with the male individual. It seems that discrimination is tactual and kinæsthetic.

Among the females captured for me were four ovigerous rock crabs, two ovigerous green crabs and two ovigerous lady crabs, all of which had evidently been fertilized some time before, for they were successfully protected from a second fertilization. For about 3 mm. around the apertures of the seminal receptacles, the shell was soft. In the openings of the vaginæ were seen two grayish vermicular plugs. These were pulled out, the operation evidently causing discomfort to the female. The plugs were curved in such a manner that they appeared to have been moulded in the vaginæ. At the internal ends were the spermatophores, emptied and flat. In order to determine if these spermatophore plugs were formed before they were visible from the outside, I dissected a female which had been killed two days after copulation, and found that the plugs were present, but that the hardened outer ends were not protruding.

Experiments were made with males of the three species under consideration by placing with them females of the same species known to be fertilized and to have protruding plugs. In no case was a male successful in his attempts to copulate. In the case of one rock crab, the female was beaten into submission and the male succeeded in touching the plugs. The discomfort caused such violent struggles on the part of the female that she was released.

The female rock crab with eggs was placed with a male of the same species, but his efforts to overcome her resistance were futile. In neither the fertilized and spermatophore plug-bearing female, or the egg-bearing female is a true passive state produced by the beating of the chelæ and ambulatory appendages of the male.

In the case of a large male and a small female, the adjustment of the abdomens is very difficult. In the case of one large male it was noted that the adjustment was not possible in the normal position, and that he turned upon his back so that the female floated above him and her abdomen was extended to a greater degree. Then he slowly drew her into position, and having inserted his verges, assumed the normal position above her. In all of the species studied, the female is not passive in the later stages of adjustment, but uses her ambulatory appendages to assist in the turning of her body. I should have liked to place a soft-shelled male with a male that had just been separated from a soft female, but was not able to do so.

3. Mating Habits of Other Arthropoda.

Crustacea.—Herrick did not study the mating habits of the lobster (16) but noted the fact that there were external seminal pouches in the female. He states that the copulation is independent of the moulting of the female. Herrick mentions the discovery by Milne-Edwards of a female *Cancer pagarus* which was fertilized and in which were what Milne-Edwards at first took to be the wands of the male, broken off after copulation as in the insects. Later, Milne-Edwards said in reference to this observation that it seemed probable that the stoppers found in the copulating pouches of the female were "of the nature of spermatophores rather than a fragment of the penis."

Holmes (17) found that in Amphipods the oviposition occurs after the moulting of the female. The males capture the females before the moult and leave them for a few hours, resuming possession of them after the moult. He found that the method of recognition is neither by sight nor smell. He removed antennæ and blackened the eyes, and still the males grasped the females. Holmes concludes that the male has the instinct to seize and carry another individual of the same species, while the female has the instinct to lie quiet when seized. He found that dead individuals were not carried and explained this by the lack of an occasional movement causing a struggle on the part of the male to retain his hold. Holmes has mentioned also the work of Leydig on *Artemia*, in which the latter found that the males of *Artemia* clasp the females by means of peculiarly shaped antennæ, and the two sexes swim about together for several days.

Dohrn (Holmes, 17) noted that the males of copepods while mating, swim about on the backs of the females much as in the Amphipoda.

Dearborn (9), Andrews (2, 3), Chidester (6, 7) and Pearse (26), working with crayfish, have shown that recognition is largely tactual. Andrews discovered the annulus ventralis in *Cambarus*, the American crayfish found east of the Rockies. This serves to retain the spermatophores until the female lays her eggs. In the crayfish, fertilization of the eggs is external. Chidester found (6) that males grasp other males and attempt to copulate with them. Pearse found that males attempt to copulate with dead females of the same and different species (26). Andrews holds that the male recognizes the female as a female because she does not resist, hence the dead or bound male is taken for a female. He says (3): "It is thus possible that from the crayfish standpoint the only difference between the sexes is a difference in behavior and not a difference in form, and moreover a difference received by muscle and touch sense and not in effect upon any other sense organs."

It seems to the writer, that although in the crayfish and the lobster, the males do not capture the females and watch over them while they moult, the process is analogous, for the freshly moulted female is soon captured and fertilized. In the case of the crayfish which have annuli, the subsequent copulations are futile on account of the presence of the hard vermicular plug deposited in the false pouches by the male. Again, in the lobster and in the European crayfish, *As acus*, although there are no pouches, the spermatophores would naturally adhere to the freshly moulted surface better than to a surface covered with accretions.

The term "recognition" has been used by Pearse and others without a proper appreciation of the fact that we have absolutely no right to assume a true r cognition in the Arthropoda. Andrews has brought out this fact in his statement regarding the crayfish, *Cambarus affinis*. He says (3): "Sex 'recognition' exists apparently, only in the sense that the male may carry out all the stages of conjugation if a female happens to be seized, but not if a male is seized."

"Recognition," it seems to the writer, should be relegated to disuse in speaking of the lower animals. The term "discrimination" may be safely used. Arthropoda may use vision, "contact-odor," kinæsthetic or other senses in *discrimination* without really "recognizing" sex.

Insecta.—Feré, in a study of cockchafers (11) found that sexual coupling failed to take place when the antennæ were removed. He also found that males which had just coupled with females proved sexually attractive to other males. It is not just certain that Feré was correct in supposing that the males were attractive to other males, because the former retained the odor femina. He found similarly in *Bombyx* that males sometimes proved attractive to other males (12).

Mayer, working with moths (21), found that the male moths find the females by smell (Forel's contact odor sense?) rather than by sight. Males flew to the abdomens of females which were placed near the detached winged bodies. Males would pair with a female minus wings, but would not approach a male with female wings. In the moths, there would seem to be a distance-odor perception.

Berlese observed that in flies, there was only a slight activity of the external organs of the male, and a great activity of the female. The male mounted the female, but the female was then the active party, introducing her ovipositor into the genital atrium of the male (5).

Maeterlinck's popular description of the life of the bee, states that the male honey bee captures the female in her nuptial flight and leaves part of his copulatory appendages in her seminal receptacles (20). The factor of natural selection is active in the mating of the bee, for the strong males are the ones to capture the females.

Fielde (13), Wheeler (34) and others mention the mating of ants. Sex-discrimination is by smell. Forel calls it "contact-odor."

Mitchell (22), worked with mosquitoes, finding that the sexes discriminate each other by the song. The female flies into a cloud of dancing males. It is possible that discrimination is *visual* at first, rather than auditory as Miss Mitchell believes.

In confinement, the female stands quietly on the floor of her cage with the male beneath and clinging with his body parallel to hers. Any disturbance which causes the female to move, when the pair are not copulating, causes the male to seize her again. The mating is repeated every hour or oftener, when the male is excited by a movement of the female. This may be brought about by a tap on the cage. When unconfined, one male unites with several females. A single fertilization lasts for five layings. Miss Mitchell concludes that the male continues to approach the female by means of the olfactory stimuli through the sensitive hairs on the antennae. Distant localization is according to her idea, by sound; discrimination of the quiescent female near at hand is by smell. Moulting as occurring before fertilization is not mentioned.

Stockard, working with the walking-stick, *Aplopus Mayeri* (32), found that males mated with artificial females made by fastening the abdomens of females to sticks and supporting the sticks on wire legs.

Arachnida.—Montgomery, 1903 (23), and Porter, 1906 (30), have summarized pretty fully the investigation made on the mating habits of spiders. Both these writers have contributed much original investigation. I shall not attempt to give all of the literature, relative to the mating habits of spiders, but content myself with a brief summary of the more important work.

The Peckhams have taken up in detail the courtship of spiders, and hold that (27) in the spiders, Darwin's theory of sexual selection is upheld.

Emerton (10) finds some variation in the ferocity of the female spiders. He states that in *Linyphia* and *Theridion*, the male and female live peaceably together in the same web and the male is not attacked by the female during his approaches.

In *Agalena* the male is the stronger of the two. He takes the female in his mandibles and lays her on one side and inserts one of the palpi; then he turns her so that she lies on the other side with the head in the opposite direction and inserts the other palpus. The female lies as though dead. Emerton holds that the method used in all of the spiders is for the male to discharge the seminal liquor on a little web spun for the purpose, and to dip the palpi in it; then he approaches the female and inserts the palpal organs into her epigynum. One palpal organ at a time is replaced by the other. Emerton believes that few males are eaten by females.

Montgomery has made most extensive studies of the mating habits of spiders (23, 24). He holds that in recognition, touch is the most important and sight is next in importance. He discredits conscious sexual selection on the part of the female.

Porter agrees with Montgomery in his statement that the female chooses that male which "first and most surely announces by his movements that he is a male." Porter found that in the *Argiope* (30) the male waits until the female has moulted, and then successfully mates with her. He found that in some cases the male left the web after approaching the female who was not ready to moult and finding her hostile. After the copulation the female wraps the male just as she does any prey, and leaves him hanging in the web with her old skin. Frequently there are as many as five male spiders on one web.

Petrunkevitch holds that sight is the only sense of sex recognition used in the hunting spiders. After sex has been recognized, courtship begins and touch is the chief means by which the male excites the female and tests her willingness to accept him (29). Petrunkevitch found that, in *Dysdera*, the male approached the female whenever she began to dig.

Nuttall and Merriam have made a study of the copulation of ticks (25). They find that there are three distinct movements in copulation. The male introduces his mouth parts into the sexual orifice of the female, dilating it and probably exciting the female; then he apposes his own sexual aperture to that of the female and expels the spermatophore, whose neck adheres to the tip of his hypostome and is then pushed into the vagina; the next movement on the part of the male is to reintroduce his mouth parts and rupture the tip of the spermatophore.

4. CONCLUSIONS.

1. In the four species of Brachyura studied, sex discrimination is tactual.

2. In the Brachyura, fertilization is effected when the female has recently moulted.

3. Males of the four species studied attempt to mate with males and fertilized females of the same species but do not attempt to mate with individuals of a different species.

4. In the Brachyura, the female is not entirely passive in the movements which precede copulation; she responds to tactual stimuli given by the male.

5. In the Crustacea, as a whole, copulation occurs at any time, regardless of the moulting of the female. It is highly probable that moulting is a necessary condition for successful fertilization.

6. In the Crustacea the female is usually passive during the act of copulation.

7. Sex discrimination in the Crustacea is kinæsthetic and tactual.

8. In the Crustacea, the males generally die soon after fertilization. A small proportion of males live for some time.

9. In the Insecta, sex discrimination is by smell; Forel's "contact-odor sense" is the fully describing term for a large degree of this discrimination.

10. Moulting has not been shown to be a necessary forerunner of fertilization in the Insecta.

11. The males of some species of Insecta die immediately after copulation.

12. In some species of the Arachnida, particularly the spiders, the females devour the males after copulation.

13. Moulting on the part of the female before fertilization is not the rule in spiders. It occurs in some genera.

14. In the ticks, the mouth parts of the male are used to moisten and stimulate the sexual aperture of the female and thus prepare the way for the injection of the spermatophore.

15. Sexual selection on the part of the female has not been definitely established in the Arthropoda. It appears that the successful male is the one who is strong enough or wary enough to mate with the female, or who has learned soonest by his senses the location of the female.

16. Since chance is a great factor in the presence of the male at the proper time, we must consider that the successful male is the one who first demonstrates his maleness to the female. Though strength is a great factor, opportuneness of proximity appears to be a greater one.

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BIOLOGICAL BULLETIN

EXPERIMENTS ON PATTERN-VISION OF THE HONEY BEE.

C. H. TURNER.

INTRODUCTION.

In my paper on the "Color Vision of the Honey Bee," published in the BIOLOGICAL BULLETIN for October, 1910, some experiments were described which, I think, demonstrate conclusively that bees can discriminate between colors. In November of that same year, J. H. Lovell[1] published the results of some researches conducted in a manner quite different from my method; but which led him to form the same conclusion.

On page 277 of my paper is the following paragraph: "How minute are the details that bees observe I am not prepared to say; but that they do observe details is indicated by the following observations: (1) In the boxes used in the experiments of Series IV., the trays of the boxes used were entered, from the portico, by means of an eccentric opening. In most of the boxes this doorway was rectangular, in others it was circular. When a bee first approached one of these boxes, she had to search for the entrance. After a few trips she would land on the portico directly in front of the entrance, and, in some cases, she would fly into the tray without even pausing on the portico. (2) For about an hour bees had been collecting honey from some of the red artifacts. It seemed that nearly all of the bees in that part of the field were collecting from those artifacts. Along side of one of the red boxes I placed a red box on the sides and top of which I had pasted bits of white paper. This gave a box with red front and spotted sides. Into this box I placed some honey. The bees that approached this box from the front always entered immediately; the majority of those that approached the sides paused a moment, then went to the nearest red box." This experiment indicated that bees could discriminate between patterns. The purpose of the researches to be described in this article was the establishment of a definite experimental answer to that question.

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[1] Lovell, J. H., "The Color Sense of the Honey Bee; Can Bees Distinguish Colors," *Amer. Nat.*, Vol. XLIV., Nov., 1910, pp. 673-692.

The method decided upon was to permit a few bees to learn that honey could be collected easier from artifacts of a certain color pattern than it could be from ordinary flowers; and, after that had been thoroughly learned, to see if those bees could select artifacts of that color-pattern from those of a different color-pattern; 1st, when the artifacts to be selected contained honey and the others did not; 2d, when not only the artifacts to be selected but some of the others also contained honey; 3d, when none of the artifacts contained honey.

The artifacts used were constructed out of cardboard and were of three kinds: circular discs about forty millimeters in diameter, cornucopias made in the manner described on page 260 of the article published last year, and special experiment boxes made in the manner described on pages 271 and 272 of that article. The artifacts used this year were smaller than those used last. The cornucopias, excluding the lip, were fifty millimeters long and thirty millimeters in diameter at the largest part. The boxes, excluding the lip, were sixty millimeters long, forty millimeters wide and twenty millimeters high. The pattern that distinguished any artifact was placed upon both the inside and the outside. The following colors and color patterns were used: plain red, plain green, alternate red and green longitudinal stripes, alternate red and green transverse stripes, alternate black and

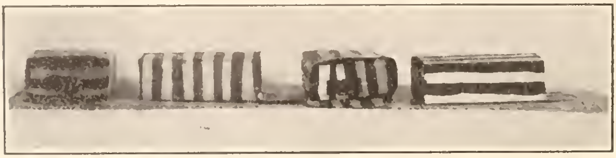

FIG. 1. A few of the artifacts. From left to right: front view of a transversely striped box, side view of a transversely striped box, front view of a longitudinally striped box, side view of a longitudinally striped box.

white longitudinal stipes, mottled red and green. With the exception of the red of some of the plain red artifacts, the red and green used on all of the artifacts was of the same hue. The stripes were about five millimeters wide; the spots on the mottled artifacts varied from two to five millimeters in length; they were irregular in shape and arranged in a haphazard manner.

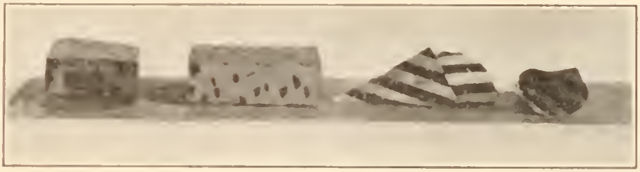

FIG. 2. A few of the artifacts. From left to right: front view of a mottled box, side view of a mottled box, side view of a longitudinally striped cornucopia, end view of a longitudinally striped cornucopia.

In order to have the bees working under normal field conditions, an abandoned meadow was selected as the site of the experiment plot. Most of the field was rank with *Melilotus alba* Lam.; but here and there were patches of almost barren ground. The majority of the experiments were performed in one of these partly barren spots, in which a few scattered mint plants and a few plants of the white sweet clover were growing.

DESCRIPTION OF THE EXPERIMENTS.

Series I. [July 14-17.]

The purpose of this preliminary series of experiments was to permit the bees to learn that honey could be obtained much more easily from artifacts marked with alternate red and green longitudinal lines than it could be from the flowers of the melilot or the mint from which they were foraging.

EXPERIMENT 1. *Early in the afternoon of July 14, two discs, marked with parallel red and green stripes and well supplied with honey, were attached to the branches of a melilotus bush from which bees were collecting honey.*

Although the discs remained on that plant all of the afternoon, no response was made to them by any of the bees.

EXPERIMENT 2. *Three discs, marked with parallel red and green stripes, were attached to melilotus twigs and a large drop of* *honey placed on each. These were placed in position at eight o'clock on the morning of the fifteenth of July.*

These discs were watched until two o'clock in the afternoon; but not a bee made any response to them.

Experiment 3. *At about eight A.M., July 17, 1911, two discs, marked with red stripes on a green back-ground were attached to the tops of upright sticks placed in the midst of a mint weed from which several bees were collecting honey. By means of a string the branches of the plant were drawn together in such a manner as to have several blossoms near each of the discs. Honey was placed on the discs and on some of the blossoms near by. Accidentally some honey fell upon some of the leaves.*

After waiting, restlessly, in the hot sun, for nearly two hours, I was rewarded for my patience by seeing a bee alight on one of the discs, and, while hanging suspended from its edge, sip the honey. When it had finished and before it started for the hive, the bee made a short flight of orientation. A few minutes later a bee alighted on the top of disc number two and began to sip the honey. Before departing for the hive, it made a flight of orientation. From this time to the close of the experiment, thirty-five visits were made to the discs, twenty to disc one and fifteen to disc two. Often bees were on both discs at the same time. On one occasion, three bees were on disc two and two on disc one. On another occasion, two bees were on each disc. Soon after the bees began to collect from the discs I noticed that several bees were collecting honey that had dropped on the leaves. To check them, wherever I saw honey on a leaf I covered it with dust. By this means I hoped to cause those bees to notice the honey on my artifacts. The habit of collecting from leaves had become fixed and those bees spent the remainder of the afternoon searching the leaves for honey. Occasionally they would find a new supply and collect therefrom until I covered it with dust. By the close of the afternoon, about six bees were collecting from the artifacts and four hunting for honey among the leaves.

Experiment 4. *About two hours before departing for home, disc two was placed in a new position and a cornucopia marked with alternate red and green longitudinal lines was placed where* *it had been. Disc one was left in its original position. All of these artifacts were well supplied with honey.*

Up to the close of the experiment, eleven visits were made to the artifacts; four to disc number one, three to disc number two, and four to the cornucopia.

EXPERIMENT 5. *Half an hour before departing for home, disc number one was placed in a new position and a second cornucopia, resembling number one in its color pattern, was placed in the place rendered vacant by the removal of disc number one. Cornucopia number one and disc number two were left in the positions occupied in experiment 4. All of the artifacts were well supplied with honey.*

The bees made twelve visits to these artifacts; four to disc number one, two to disc number two, three to cornucopia number one, and three to cornucopia number two.

Experiment number three was begun at about eight A.M., experiment number five closed at five P.M. Throughout the whole of that interval the artifacts were observed continuously.

On leaving for home all of the artifacts were well supplied with honey and left in the field.

The bees collecting from those artifacts were only a small per cent. of those visiting that part of the field; but they behaved so unlike the others that I could recognize one as soon as it arrived within a yard of the artifacts. The bees that had not begun to collect honey from my artifacts or honey-smeared leaves, on reaching the experimental plot, would simply wander from flower to flower, pausing a moment at some and simply glancing at others. On the other hand, bees that were collecting honey from my artifacts or honey-smeared leaves, on reaching the plot, would describe a number of complex, irregular, number eight curves, no two of which seemed to be in the same plane and which became gradually shorter and shorter until the artifact or honey-smeared leaf was found by the bee. To one looking on, it was almost impossible to resist the belief that those bees were seeking some definite thing or things.

Series II. [July 18, 1911.]

On reaching the field, at about eight thirty A.M., there was no honey in any of the artifacts; but the bees were moving in and out of the cornucopias as though they were hunting for something.

EXPERIMENT 6. *One of the cornucopias used in the last experiment was replaced by an experiment box marked with alternate red and green longitudinal stripes. The other cornucopia was left in place and called striped cornucopia number one. Three groups of three short stakes each were erected near the plant containing the two stakes mentioned above. The groups were about a foot apart and the stakes in each group separated by an interval of about two inches. The groups were numbered from two to four, two being the nearest to the two stakes and four being the most remote. The stakes were arranged in a section of a curve. On the top of one of the stakes in each group a cornucopia marked with alternate red and green stripes was placed, while a similar plain red and plain green cornucopia crowned each of the other stakes. In no two groups was the arrangement of the cornucopias the same. In the striped box and in the striped cornucopias, honey was placed. The other artifacts were empty. The position of the artifacts remained the same throughout the experiment.*

This experiment began at about nine A.M. and extended until noon. Except during the time consumed in taking a photograph, the artifacts were watched continuously. Not a single visit was made to either the red or the green cornucopias; but forty-seven entrances were made into the cornucopias that were marked with alternate red and green longitudinal stripes. These visits were distributed as follows: striped box number one, nine; striped cornucopia number one, twenty-one; striped cornucopia number two, eight; striped cornucopia number three, seven; striped cornucopia number four, two. Thus all of the striped artifacts were visited. Sometimes a bee would enter one artifact and then leave and enter another and sometimes yet another. It usually sipped honey in the last visited artifact only. In some cases the cause of leaving the first artifact was the presence of some bee; in most cases, however, I could not discover the cause for departing.

EXPERIMENT 7. *A fourth group of three stakes was added to the above group and called group five; it was arranged about half way, in a straight line, between group two and group four. All of the* *cornucopias were replaced by experiment boxes of the same color patterns. At irregular intervals, the arrangement of the boxes in each group was altered. All of the boxes marked with alternate green and red longitudinal stripes contained honey, the other artifacts were empty.*

This experiment extended from noon to five P.M. Except while taking a photograph, the artifacts were watched continuously. During that time not a single entrance was made into either the green or the red boxes; but eighty-nine entrances were made into the striped boxes; and each such box was visited several times. The visits were distributed as follows: striped box number one, eight; striped box number two, fifteen; striped box number three, twelve; striped box number four, seven; striped box number five, twenty-one.

One of the cornucopias used in this experiment leaked and formed, on the slightly inclined stake, a line of honey. Several bees discovered this honey and began to carry it to the hive. The line of honey was on the side of the stake that was turned away from me and I did not know that the bees had formed the habit of collecting honey from the side of the stake until experiment seven was half over. I saw bees flying to that side of the stake; but I supposed they were some of the bees who had formerly formed the habit of collecting honey from smeared leaves, and, since I had seen to it that there was no honey on any of the leaves, I did not give them much attention until I noticed that bees were frequently flying from that place to the hive. As soon as I realized that they had formed that habit, I covered the honey with road dust, hoping to induce the bees to attend to my artifacts. Instead of entering the artifacts, they continued to examine the stake; occasionally one would fly to another stake and examine it. This was not because they had not noticed the artifacts, but because the artifacts had acquired no meaning to them. They would frequently approach one of the boxes containing honey and hover before it momentarily and then return to searching the stakes. To them the stakes had come to mean "honey-bearers" and they seemed to use the artifacts and neighboring plants as reference points.

Most of the bees continued to describe those complicated number eight curves, whenever they arrived at the experiment plot; but the number of curves described were perceptibly fewer and the lengths much shorter, and some of the bees seemed to fly practically direct to the artifacts.

On departing for home, all of the artifacts, except three striped boxes, were removed. Those boxes were well supplied with honey.

July nineteenth, I arrived at the field at 9:30 A.M. and found the boxes empty and the bees flying into and out of them as though hunting for something. It began to rain soon after my arrival, which made it impossible to continue my experiments that day. On leaving, all of the artifacts were removed from the field.

Series III. [July 20, 1911.]

The morning was quite cloudy and when I arrived in the field, at about half past seven, the bees were not foraging.

EXPERIMENT 8. *Three groups of three experiment boxes each were arranged in the manner described above. In each group there was one box marked with alternate red and green longitudinal stripes, one green box and one red box. The order of the boxes was different in each group. When the experiment was about half over, the arrangement of the boxes in each group was changed. All of the boxes marked with alternate red and green longitudinal stripes contained honey; the others were empty. The experiment was an hour long.*

Twenty-five entrances were made into the interior of the striped boxes; but not a single entrance into either the red or the green boxes. The visits were distributed as follows: striped box number one, ten; striped box number two, eight; striped box number three, seven. Only once did a bee, on arriving at the plot, make the irregular number eight curves mentioned above. On arriving at the plot, the bee would either fly directly to one of the striped boxes, or else fly along, examining box after box, until it reached a striped box. On leaving, the bees did not always make flights of orientation. Often a bee would arrive at the hind end of one of the experiment boxes that contained honey and attempt to find an entrance; then it would sidle around to the front end and enter.

EXPERIMENT 9. *Three groups of boxes were arranged as in* *experiment eight; but honey was placed, not only in the striped box, but also in red box number one and green box number two. When the experiment was about half over, red box number one was placed where striped box number one had been and striped box number one placed on the stake made vacant by the removal of red box number one; and green box number two was placed where striped box number two had been and that striped box placed on the stake made vacant by the removal of green box number two. The time occupied was about one hour.*

Twenty-two entrances were made into the striped boxes, half before and half after the change was made in the arrangement of the boxes; but not a single visit was made into the interior of either the red or the green boxes, although one box of each of those colors contained honey. The visits were distributed as follows: striped box number one, thirteen; striped box number two, four; striped box number three, five. One bee hovered before the hind end of red box number one for several seconds and then flew to striped box number one. Another bee alighted on the front end of red box number one and then departed. Bees often paused momentarily before all of the boxes; but they never entered any that were not marked with alternate red and green longitudinal stripes.

EXPERIMENT 10. *Three groups of boxes were arranged as in experiment eight; but no honey was placed in any of the boxes and none had ever contained honey. The duration of the experiment was about fifteen minutes.*

Fourteen entrances were made into the striped boxes; but not a single visit was made to the interior of either the green or the red boxes. The visits were distributed as follows: striped box number one, six; striped box number two, three; striped box number three, four.

EXPERIMENT 11. *Two groups of artifacts were removed from the field; thus leaving only one group of boxes, all of which were empty and none of which had ever contained honey. The time allowed was about ten minutes.*

Ten entrances were made into the striped box, but not a single visit was made to the interior of either of the other boxes. When I terminated the experiment, about six bees were hovering about the striped box, examining it carefully.

Series IV. [July 21, 1911.]

EXPERIMENT 12. *Two sets of five boxes were arranged on stakes. In each group there was one of each of the following artifacts: box marked with alternate red and green longitudinal stripes, box marked with alternate black and white longitudinal stripes, box mottled with green and red, a plain red box, and a plain green box. The arrangement of the boxes in each group was different, and was changed twice during the experiment. The box marked with alternate red and green longitudinal stripes was supplied with honey; the others were empty. The time consumed was about one and a half hours.*

Sixty-three entrances were made into the boxes marked with alternate red and green longitudinal stripes and one into the boxed mottled with green and red; no visits were made into the interiors of any of the other boxes. Bees frequently hovered before other boxes and twice bees alighted on the portico of the box marked with alternate black and white longitudinal stripes, but did not enter. The visits to the artifacts marked with alternate red and green longitudinal stripes were distributed forty-two to box one and twenty-one to box two.

EXPERIMENT 13. *One set of six boxes was arranged on stakes. To the kinds of boxes used in experiment 12, I added a box marked with alternate red and green longitudinal stripes. The box marked with longitudinal red and green stripes was supplied with honey; the others were empty. Starting at the left, the arrangement of the boxes was as follows: plain green, marked with alternate red and green longitudinal stripes, mottled with green and red, marked with alternate black and white longitudinal stripes, plain red, marked with alternate red and green transverse stripes. The time consumed was about half an hour.*

Nineteen entrances were made into the box marked with alternate red and green longitudinal stripes; but no visits were made to the interior of any other box.

EXPERIMENT 14. *The same number and kinds of boxes were used as in experiment thirteen; but the mottled red and green box was placed where the box containing honey had been and the box containing the honey was placed on the stake rendered vacant by the removal of the mottled box. The time consumed was about half an hour.*

Twenty entrances were made into the box marked with alternate red and green longitudinal stripes and one into the box that was mottled with red and green. No visits were made into the interior of any other box.

EXPERIMENT 15. *The same number and kinds of boxes were used as in experiment fourteen; but the box marked with alternate red and green transverse stripes was placed where the box containing the honey had been, and the box containing the honey was placed where the box marked with alternate red and green transverse stripes had been. The time consumed was about three-quarters of an hour.*

Thirty-six entrances were made into the box marked with alternate red and green longitudinal stripes, six into the box marked with alternate red and green transverse stripes, and two into the box marked with alternate black and white longitudinal stripes. No visits were made into the interior of any other box. The bees that selected the wrong boxes did not enter them as promptly as did those that entered the right box. In each case the bee hovered before the entrance quite a while before entering. All of these mistakes were made during the first ten minutes of the experiment.

EXPERIMENT 16. *Two boxes marked with alternate red and green longitudinal stripes and two boxes marked with alternate red and green transverse stripes were arranged on stakes, in a line, with all of the entrances facing in the same direction. For the first half of the experiment the different kinds of boxes alternated; during the latter half, the boxes with the longitudinal stripes were in the middle. The boxes with longitudinal stripes contained honey, the others were empty. The time consumed was about half an hour.*

The purpose of this experiment was to accustom the bees to ignoring the transversely striped boxes. Thirty-three entrances were made into the longitudinally striped boxes and none into any of the others.

EXPERIMENT 17. *The same number and kinds of boxes were used as in experiment fifteen and they were arranged in the following order: box marked with alternate black and white longitudinal stripes, box marked with alternate red and green transverse stripes, box marked with alternate red and green longitudinal stripes, plain green box, box mottled with red and green. The box marked with* *longitudinal red and green stripes contained honey; the others were empty. The time consumed was about fifteen minutes.*

Eleven entrances were made into the box marked with alternate red and green longitudinal lines; no entrances were made into any of the other boxes.

EXPERIMENT 18. *The same kind and number of boxes were used as in the above experiment, but they were arranged in the following order: box marked with alternate black and white longitudinal stripes, box marked with alternate red and green transverse stripes, plain green box, box marked with alternate red and green longitudinal stripes, box mottled with red and green, plain red box. None of the boxes contained honey or ever had contained honey. The time consumed was about fifteen minutes.*

Thirty entrances were made into the box marked with alternate red and green longitudinal stripes, but no visits were made into the interior of any other box.

EXPERIMENT 19. *An empty box marked with alternate red and green longitudinal stripes and which never had contained honey was placed on the palm of my hand and by its side was placed an empty box marked with one of the other patterns. The entrances of both boxes were made to face the same way. All other artifacts were removed from the field and my hand held near the stakes upon which artifacts had rested. At the end of about two minutes the position of the boxes was altered. This maneuver was repeated with each kind of box used in experiment eighteen.*

At least a hundred entrances were made into the box marked with red and green longitudinal stripes; but not a bee entered any of the other boxes. One bee acted as though it was going to enter the box marked with alternate red and green transverse stripes, but it did not do so. Towards the end of the experiment the bees entered the box marked with alternate red and green longitudinal stripes so rapidly that it was almost impossible to keep an accurate record. To one looking on, it seemed as though the bees were looking for something which they expected to find in those boxes.

When I left for home the bees were searching over and around the barren stakes for something that was not.

INTERPRETATION OF THE EXPERIMENTS.

These experiments furnish additional evidence, if such is needed, that Plateau is mistaken when he claims that odor is the sole factor that leads bees to the honey of flowers; for my discs, marked with red and green stripes and well supplied with honey, remained exposed to bees for fully two days before a single bee collected honey from them [Ex. 1–5].

These experiments strengthen the impression, gained from previous work with hymenopterous insects, that such insects can be induced to form new associations that will influence their behavior, if only the experiment is so planned as to lead the insect to accidentally make the association. So far as my experience goes, it is almost impossible to coerce them into forming new associations, and an abundance of time is often required for the formation of chance associations. Lack of response to a stimulus does not mean that that stimulus has not been noticed by the insects; but that, to them, it has not yet acquired a meaning.

In inducing bees, in the field, to respond to new food-objects, the new associations are formed much more quickly when the food is exposed to view than when it is hidden. They may be induced to form such associations, however, even under the latter conditions. These statements are supported by the fact that last year, when I conducted a lengthy series of experiments with discs and cornucopias, artifacts which did not hide the honey from view, I soon had more bees responding to the artifacts than I could keep an accurate record of; whereas this year, when I laid aside my discs and cornucopias as soon as about half a dozen bees had made the association, at no time were more bees at work than could be counted. At the close of the series about twice as many bees were collecting from the artifacts as at the beginning.

When a bee had discovered one of my honey-producing artifacts and collected therefrom, it would make a flight of orientation and then fly home. On returning to the field it would, by means of a series of ever narrowing, complex, figure eight curves, search out the experiment plot, and then, by a closer scrutiny of the surrounding objects, locate the artifact desired. [Last paragraph of Series I.] After a bee had made several trips to the plot, it would fly directly to it without making the complicated searching movements. This striking behavior furnished a sure means of telling when a new bee had formed the association. I changed the arrangement of the individual artifacts so often that the bees usually were forced to search for the honey-producing box. After the association had been well established, the bees usually departed for home without making a careful flight of orientation. If, however, I had made a marked change in the position of the artifact since the last visit of the bee, then a careful flight of orientation was always made. No one who had watched these pronounced searching movements could doubt that bees are largely guided by memory pictures of the environment.

After a bee had learned, by experience, that artifacts bearing a certain color pattern contained a more copious supply of easily obtained honey than ordinary flowers, it would select artifacts bearing that color pattern from those marked in a different way. This was true: (1) when several of the artifacts to be selected were scattered among a number of plain artifacts of the colors used in making the color-pattern [Ex. 6–11]; (2) when the artifact to be selected was scattered among several other artifacts, some of which were plain and some of which were marked with patterns unlike that of the artifact to be chosen [Ex. 12–15, 17, 18]; (3) when the only difference between the artifacts was that one was marked with transverse and the other with longitudinal stripes [Ex. 16]; (4) when the artifact to be selected contained the honey and the others did not [Ex. 8, 12–17]; (5) when honey was to be found not only in the artifact to be selected, but in some of the other artifacts also [Ex. 9]; (6) when none of the artifacts contained honey [Ex. 10, 11, 18, 19].

All of the facts mentioned in the above paragraph are recorded in the following table.

Although this table shows conclusively that bees can recognize color patterns it does not tell the whole story. It does not show that two of the mistakes were made in selecting a box mottled with red and green for one marked with longitudinal red and green stripes; that two mistakes consisted in selecting a box marked with black and white longitudinal stripes for one marked with

TABULATED RESPONSES OF HONEY BEES TOWARDS PATTERNS.

The bee to select an artifact marked with alternate red and green longitudinal stripes when associated with	No. of Trials.	No. of Successes.	No. of Failures.	Per Cent. of Successes.
Plain red and plain green artifacts, an equal number of the three kinds being used; when the artifact to be selected contained honey and the others did not.	161	161	0	100.0
Plain red and plain green artifacts, an equal number of each of the three kinds being used; when the artifacts to be selected and some of each of the other kinds contained honey.	22	22	0	100.0
Plain red and plain green artifacts, an equal number of each of the three kinds; none of the artifacts contained honey. .	24	24	0	100.0
Artifacts marked with alternate red and green transverse stripes; the artifacts to be selected contained honey, the others were empty.	33	33	0	100.0
Two of each of the following artifacts: plain green, plain red, mottled red and green, marked with alternate black and white longitudinal stripes; the artifacts to be selected contained honey, the others did not	64	63	1	98.5
One of each of the following artifacts: plain green, plain red, mottled red and green, marked with alternate black and white longitudinal stripes, marked with alternate red and green transverse stripes; the artifact to be selected contained honey, the others were empty.	84	75	9	89.3
One of each of the following artifacts: plain green, plain red, mottled red and green, marked with alternate black and white longitudinal stripes, marked with alternate red and green transverse stripes; none of the artifacts contained honey	30	30	0	100.0
Any one of the artifacts mentioned in the rectangle immediately above this one; the bees being given an equal number of chances at each of the five pairs; none of the artifacts contained honey.	100	100	0	100.0
Grand total	518	508	10	98.5

red and green longitudinal stripes, and that the other six mistakes consisted in selecting a box marked with red and green transverse stripes for one marked with red and green longitudinal stripes. And it does not show that the bees soon learned to avoid making even these mistakes.

There was much in the behavior of these bees to indicate that, in their ability to distinguish minute details, they are near sighted. On examining the artifacts, either when searching for an artifact or in making its flight of orientation, the bee always hovered within about a centimeter of the object examined. The first box a bee reached, on arriving at the plot, was usually not the desired one, and, after a momentary examination, it would pass on to the next.

Evidently bees can distinguish between color-patterns, and this is of value to them in recognizing plants that yield honey. Hence, since insects can distinguish colors and the fine details of color pattern, there is nothing about the visual powers of bees that militates against the theory that the colors and the color markings of flowers are adaptations to insect visitors.

Summer High School,
St. Louis, Mo.,
July 22, 1 11.

PHOTOTACTIC REACTIONS AND THEIR REVERSAL IN THE MAY-FLY NYMPHS HEPTAGENIA INTERPUNCTATA (SAY).

J. E. WODSEDALEK.

REACTIONS TO LIGHT.

When nymphs of the may-fly *Heptagenia interpunctata* are placed in a long glass dish of water near a window, they immediately swim away from the light, and upon reaching the end of the dish they repeatedly collide with and claw against the invisible obstruction. These movements may be kept up, with intervals of rest, for a whole day. When quiet, the nymphs do not always face away from the light, nor when they swim do they always go in a straight line away from it. Often if there are large dark objects in the room, they will swim against the side of the dish in the direction of the objects.

As the behavior of the nymphs is often much affected by objects in the neighborhood, most of the experiments on the reactions to light were performed in a dark room in a large, evenly colored box constructed of white cardboard, thus shutting off all objects and undesirable light. The light usually employed was derived from an ordinary sixteen candle power incandescent lamp attached to a wire and movable about at will. Almost without exception, the nymphs swam away from the light. Occasionally, some specimens after repeatedly clawing against the transparent barrier and not securing a hold would fall down on their backs with their legs sticking up, remaining in that position sometimes for hours. If a stone was placed in the end of the basin nearest to the light, the nymph would attach itself and remain there for days. When the light was brought near the stone and moved about, the insect would seek the shaded area, but light even much more intense would seldom force it to desert the stone entirely.

When a piece of white cardboard is placed on the water in a small glass dish, the nymphs soon attach themselves to the lower side of it. By illuminating that side of the paper they soon crawl upon the top which is shaded. Then, when the strong light is again thrown from above, they move below, and in that manner can be made to move back and forth repeatedly, though not with such precision after the experiment is kept up for some time. The nymphs respond to light most readily when occupying the upper side of the paper, and the best results are obtained when white paper is used. Quite similar results are obtained by using gray or black paper, but in such cases the nymphs take a much longer time to move from one surface to another.

I have performed quite a number of series of experiments with different groups of nymphs using two lights, differing in intensity, one at each end of a glass basin twenty-four inches long. I noticed that by placing the nymphs in the water half way between the two lights they would often continue going in the direction in which they first started, regardless of the intensity of the light, though many of them manifested much promptitude in going towards the end of the dish from whence came the light of less intensity, even when there was a difference of only a few candle powers. The excitability apparent in many specimens at the instant of being placed in the water, which is largely responsible for their reckless behavior was almost totally reduced by placing the nymphs in a narrow tunnel made of glass strips, which was placed in the middle of the large basin at right angles to the rays of the two lights. The tunnel was sufficiently wide to enable the specimens to go forward, but not wide enough to enable them to turn around in it. Then the nymphs were put in at one end and allowed to pass through two or three inches of the tunnel, thus getting the full force of the two lights at the same time, and also a chance to resume a more normal attitude. By the time the end of the tunnel was reached, which was in line with the two lights, it was only a matter of choice which direction was to be taken. Sometimes the nymphs would manifest no choice, but remain in the middle of the dish for several minutes, especially when the two lights differed little in intensity. But, generally the insects evinced considerable precision in choosing the end of the basin from which came the light of less intensity. The results obtained in some of the experiments are given in the following tables.

TABLE I.

Series.	Turns Toward 80 c.p.	Indifferent.	Turns Toward 16 c.p.	Total.
1	0	0	10	10
2	0	0	10	10
3	0	0	10	10
4	0	0	10	10
5	0	0	10	10
6	0	0	10	10
Totals	0	0	60	60

TABLE II.

Series.	Turns Toward 32 c.p.	Indifferent.	Turns Toward 16 c.p.	Total.
1	0	2	23	25
2	0	0	25	25
3	1	3	25	25
4	2	0	23	25
5	0	0	25	25
6	1	1	23	25
Totals	4	6	140	150

TABLE III.

Series.	Turns Toward 10 c.p.	Indifferent.	Turns Toward 16 c.p.	Total.
1	1	2	7	10
2	1	3	6	10
3	0	3	7	10
4	0	1	6	10
5	2	3	5	10
6	1	3	6	10
Totals	5	18	37	60

In further experiments an apparatus essentially the same as that used by Strasberger in his work on *Protista* was employed. It consisted of a prism of a solution of India ink placed over a long glass trough the sides and bottom of which were covered over with white paper. Ten specimens were placed in the end of the trough under the thin portion of the prism, and when fairly quiet the light was reflected perpendicularly through the prism giving a perfect gradation in the intensity of light from one end of the trough to the other. Three of the specimens immediately swam toward the dark end of the trough; four others followed within half a minute; and the remaining three collected into a group remaining that way for a few minutes when they were separated, and within another minute also left for the dark area. Sometimes a specimen would return to the less obscure end of the dish and not finding anything favorable to its thigmotactic inclination, it went back whence it came from. This experiment was repeated several times, always with similar results.

The light was next permitted to enter the trough obliquely, the thicker end of the prism being next to the light. Again five of the individuals made for the darker end of the trough almost immediately, this time going against the rays of the light. About half a minute later two other specimens followed and within three minutes all had deserted the light area. This experiment was repeated many times with ten different sets, and always in less than one minute a large majority of the specimens were in the dark portion of the trough. There were almost always a few slow specimens which required a longer time, from one to ten minutes and even much longer, to be moved. If left in the trough, almost invariably at the end of a few hours the nymphs, with some exceptions, would gather into a group in the obscure region and remain there indefinitely. If the prism and light were reversed, leaving the entangled colony undisturbed in the more illuminated end, the colony would slowly break up and within several hours again form in the more obscure territory.

Another apparatus was so arranged that the length of the prism could be increased or lessened by tipping the trough holding the solution, at various angles, and thus augmenting or diminishing the contrast between the two ends of the trough. It was observed that the proclivity of most nymphs to choose one end of the dish in preference to the other varied directly in proportion to the contrast in the illumination of the two extreme regions of the trough. Here again, we find that some individuals react far more readily than others.

It was also observed that some nymphs under ordinary light manifest no signs of agitation when in a uniformly lighted area, yet when an area in their neighborhood becomes slightly shaded they soon aggregate there.

Control of Phototactic Reactions by Chemicals.

It was found in the experimental work on the reactions to light that *H. interpunctata* nymphs are practically all negatively phototactic, but that the different individuals vary in the intensity of their negative response. Also, out of about five or six hundred specimens several were quite indifferent, and a few were even weakly positive. This obvious variability in response among the different individuals, in an identical external environment, is apparently due to the variability in the internal conditions of the various specimens.

Loeb[1] says that in all probability light produces chemical changes in the eye or skin of the animals, and that these changes are responsible for the heliotropic reactions. In a brief preliminary communication[2] on the control of heliotropic reactions in fresh water crustaceans by chemicals, he says that specimens of *Gammarus pulex*, which are naturally negatively heliotropic can be made positive by specific chemical substances. For example, if carbon dioxide is allowed to bubble through the water, or if the animals are thrown into water previously charged with carbon dioxide, they at once become positively phototactic. The same results were obtained with other acids, as hydrochloric, oxalic and acetic, but boracic acid produced no such effect. Among the salts, he found that only those of ammonium could be compared in their effects with those of the acids.

Jackson[3] experimented with another amphipod, *Hyalella knickerbockeri*, which is also negatively phototactic, and obtained results similar to those of Loeb, except, that *Hyalellas* were made positive by boracic acid. Tartaric acid, however, produced no change in their reaction. With the salts and alkalies it was again found that there seemed to be no relation between the class of chemical used and the reactions.

Mast[4] found that *Arenicola* larvæ which are normally positive, become negative in solutions containing various narcotics, acids, alkalies or neutral salts, and he claims that the sense of the reaction in these forms seems to be due to the general state of the organism as a whole, rather than to the specific effect of acid or other solutions on the chemical state of some postulated substance.

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[1] Loeb, J., "Comparative Physiology of the Brain and Comparative Psychology," 1910.

[2] Loeb, J., "The Control of Heliotropic Reactions in Fresh-water Crustaceans, by Chemicals, especially CO_2" (University of California Publications), "Physiology," Vol. 2, p. 1, 1904.

[3] Jackson, H. H. T., "The Control of Phototactic Reactions in *Hyalella* by Chemicals," *The Journal of Comparative Neurology and Psychology*, Vol. 20, No. 3, pp. 259–263, 1910.

My experiments on the effects of chemicals on phototaxis in *H. interpunctata* nymphs were performed in a dark room and the source of light was an incandescent bulb of seventy-five candle power. In each experiment twenty fresh specimens decidedly negative in their reaction to light were put in a long and narrow glass basin, three and a half by twenty-six inches, of about 2,000 c.c. capacity half filled with distilled water. The concentration of the solution was then slowly and gradually increased by adding constant small amounts of the given chemical at about ten or twelve minute intervals. A careful record was kept of the number of insects positive and the number negative at the different concentrations throughout the experiment. It was noticed that as the concentration of the solution was increased there was a gradual increase in the number that became positive. For example, in the experiment with hydrochloric acid at .01 per cent. solution, of the twenty specimens four became positive; at .02 per cent. eight were positive; at .03 per cent. fifteen were positive; and at .04 nineteen were positive. It was the same with the other chemicals employed. As a rule, specimens that once became positive remained positive throughout the experiment, and usually the insects that seemed to be the least annoyed by the light in fresh water were the first to manifest their positive phototaxis in the solution. In general the specimens that evinced their dislike for the light most obviously in fresh water were the ones that made the most frantic efforts to get at the light when they once became positive. However, the specimens as a rule were very persistent in their normal reaction, remaining negative until death occurred.

Of the different classes of chemicals employed the acids were the most effective in reversing the phototaxis of *H. interpunctata* nymphs. A large majority, and in some cases all of the twenty specimens became positive in the acid solutions. When CO_2 was bubbled through the water, again, a large percentage of the specimens became positive. The concentrations sufficient for this effect with the different acids used are,—hydrochloric .04 per cent., tartaric .04 per cent., nitric .03 per cent., sulphuric .03 per cent., boracic .05 per cent., and acetic .07 per cent. Similar results were obtained with the salts, although the number of specimens which became positive was somewhat smaller. However, all the salts employed effected a reversion, and the concentrations necessary to do this are as follows: potassium iodide .3 per cent., potassium chloride .3 per cent., ammonium bromide .3 per cent., sodium chloride .3 per cent., sodium sulphite .06 per cent., and sodium sulphate .08 per cent. The alkalies were less effective, only about half of the specimens becoming positive in these solutions. In general all of the specimens became quite inactive, and when specimens which failed to manifest a positive response were placed in the end of the basin nearest to the light, they seldom made any effort to get away. All three of the alkalies, sodium hydroxide, potassium hydroxide, and ammonium hydroxide, which were employed, produced similar results at about the same concentration, which was .09 per cent. Alcohol, too, at a concentration of about .8 per cent. produced a reversal of the reaction in a large percentage of the specimens.

In their natural environment these insects live on the under side of stones and even in the aquaria I have never seen them on the upper surface. When a certain amount of any of the above mentioned chemicals was added to the water in the aquaria, the nymphs would soon crawl up on top. The acids, again, were particularly effective in this change, and although ordinary daylight was sufficient to bring the nymphs up, the results became more obvious when the aquaria were taken in the dark room and a strong light was suspended over them.

It gives me pleasure here to acknowledge my indebtedness to Prof. S. J. Holmes, for his help and kindly criticisms.

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A PRELIMINARY NOTE ON THE RELATION OF NORMAL LIVING CELLS TO THE EXISTING THEORIES OF THE HISTOGENESIS OF CONNECTIVE TISSUE.

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The connective issue of the adult is in the embryo derived from the mesenchyme. The same is true of certain other tissues, endothelium, muscle, and cartilage, bone and dentine, if these last be not included within the scope of the term connective tissue. It is in this narrower sense that the term connective tissue is herein used.

In considering the origin of connective tissue one has to take into account two distinct elements, cells and fibers. Of the cells there are at various stages in the embryonic mesenchyme three distinct types: (*a*) the wandering cells, viz., leucocytes, which are more directly concerned with the processes of hæmatopoiesis; (*b*) those cells which give rise to the true wandering connective tissue cells of the mature tissues, the mast cells, plasma cells, etc., whose history is more or less closely concerned with that of the embryonic leucocytes; (*c*) those typical connective tissue cells which are related to the fibers and which are commonly known as the "fixed" connective tissue cells. It is without doubt only the last type which is concerned with the origin of the connective tissue fibers, hence the present note deals only with this type of cell.

At least two types of fibers have also to be considered as arising in the connective tissue mesenchyme, the elastic and the collaginous. We are here concerned only with the latter type, for the reason that the elastic fibers arise at a later period; thus the origin of the connective tissue fibers concerns primarily the collaginous, or so-called "white fibers." It is unnecessary to distinguish at the early stage under consideration between such varieties of collaginous fibers as "reticulum" and "fibroglia," for the former undoubtedly arises in exactly the same manner as the ordinary "white fibers," and of the histogenesis of the latter little or nothing is known if, indeed, it can be considered as an entity distinct from the other connective tissue fibers.

The numerous theories which have been proposed to account for the origin of the connective tissue fibers may be reduced to three chief divisions:

1. The embryonic connective tissue or mesenchymal cells become directly transformed by elongation into connective tissue fibers.

2. The fibers arise in the ground substance between the cells either by transformation of that ground substance or as a secretion from the adjacent cells.

3. The fibers arise within the cells either as distinct fibers (Schwann, 1839), as granules which fuse to form fibers (Spuler, 1896; Lavini, 1909), as an epicellular protoplasmic fibrous layer (Lwoff, 1889), or as an ectoplasm about the cell (Hansen, 1899) or forming the syncytium (Mall, 1902).

The theory of direct transformation by elongation of cells into fibers may well be abandoned and, except it be construed along the lines of the various theories of intracellular origin, it is worthy only of passing notice. Among other things the existence of an overwhelming number of fibers relative to the number of cells present in connective tissue argues against direct transformation, and modern microscopical methods are not able to detect the phases of such transformation either in normal growing tissues or in the process of wound repair. For several decades the results of observations have been in support of either the intercellular or the intracellular group of theories.

To Henle (1841) we owe the theory of the intercellular (extracellular) origin of connective tissue fibers, the fibers being supposed to arise from the intercellular ground substance, not directly from the cells. Latterly this theory has received but little support unless it be from the observations of Merkel (1895), who found that in the umbilical cord fibers appear first to arise in locations relatively remote from the cells. Later the cells wandered into these fibrous areas, and at a still later period the fibers had so increased in numbers that they necessarily laid in relation to the cells.

The weak point in Merkel's deduction appears to be the disregard of the locomotive properties of mesenchymal or young connective tissue cells. If these cells, except for the incidents of mitosis, are stationary, then Merkel's observations would render conclusive evidence to support the theory of the extracellular origin of fibers. But that such cells possess at least a limited power of motion simulating the amœboid character has long been known. An adequate theory must account for the activity of these cells. Merkel's theory presupposes them to be stationary till fibers have appeared and only later to acquire amoeboid characters.

In searching for a tissue which would offer opportunity for the study of the activities of connective tissue cells in the living animal under normal conditions, I have found that the median fin of young fish embryos affords a subject for study which throws some light on Merkel's observations.

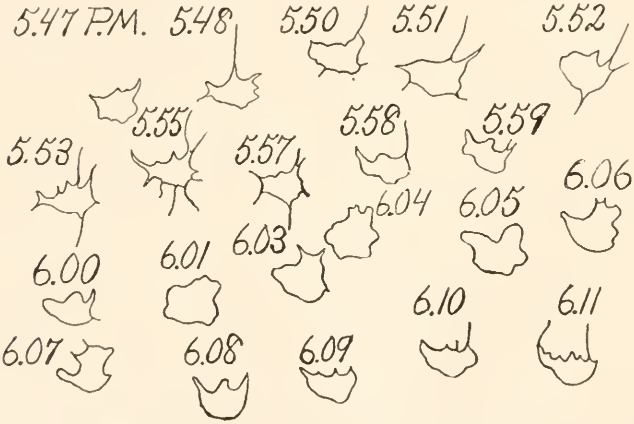


FIG. 1. Outlines of a stellate connective tissue cell in the caudal fin of a *Fundulus* embryo 4.5 mm. long (one day after hatching). The figures record the moment of completion of each drawing, the whole observation extending over a period of 24 minutes. The fish was afterward returned to water and swam for two days before being killed for further histological use. The cell observed corresponds in appearance with that shown by W. Flemming (*Arch. f. Anat.*, 1897, Fig. 9, Plate VI.) in the mesentery of a *Salamander* larva. It shows active amœboid motion and extensive changes of form. Its locomotion was not recorded, it was quite limited. × 800, camera lucida.

In a *Fundulus* embryo of 6 mm. total length—several days after hatching—I find in the median fin between the double layer of cutaneous epithelium the first signs of the dermal fin-rays. Blood-vessels have not entered, and between the fin-rays are few if any connective tissue fibers, certainly none in the distal portion of the fin. Invading the base of the fin is a mass of mesenchymal cells, mostly of the round cell type. Scattered through the fin are, here and there, isolated connective tissue cells in very limited numbers, distributed over an area representing the proximal one half or two thirds of the fin, and forming a sort of skirmish line, as it were, in advance of the army of round cells. These stellate cells can often be distinctly seen in the living animal. Later, connective tissue cells in abundance wander into the fin and fibers appear. But it cannot be said that the fibers appear in advance of the cells, for the "skirmish line" precedes the appearance of fibers, these advance cells becoming later intermingled with those of the subsequent invasion.

The advance cells, like all other stellate connective tissue cells in the fins of embryo fishes, I find to be actively amœboid, capable not only of motion but to some extent of locomotion. Since these cells are travelling through an area in which fibers are only just beginning to appear, the location of early fibers at points relatively distant from the cell in "fixed" tissue at once loses its significance and thus robs the theory of the extracellular origin of fibers of one of its strongest supports. My observations may be readily verified on any living, free-swimming *Fundulus* embryo within the first week after hatching.

Connective tissue fibers may be supposed to arise as a product of secretion of the mesenchymal cells, the extracellular deposition of fibers thus occurring under the influence of the cells. This is but a slight deviation from the strict extracellular origin presupposed by the theory of Henle and Kölliker. Such secretion must appear either in fluid form or as granules. In either case it is difficult to conceive why the secretion should take on a linear form if the cells remain stationary, and still more difficult if the cells are in motion, unless that motion be extremely slow and in one direction only.

By observation of the fins of embryo fish, chiefly *Fundulus*, I have been able to observe that the spindle cells of embryonic connective tissue possess locomotion and move largely in one direction, but their motion and locomotion is extremely active, and so far as I can observe in the living animal they leave behind no trail of secretion, certainly no observable granules: still more important, the fibers appear to have been formed in great numbers prior to the appearance of the type of spindle cells in any considerable proportion relative to the other types of cells, round and stellate, already present in the primitive connective tissue. Hence I cannot conceive of the spindle cells as producing fibers by direct secretion.

But it is at the time of the predominance of stellate cells that the fibers make their first appearance in the fish's fin, as is likewise the case in the tissues studied by other observers. These stellate cells rapidly change their form, throwing out and retracting their processes in rapid succession, as can be seen in any living, free-swimming *Fundulus* embryo under 25 mm. total length. Fig. 1 shows the outline of such an early connective tissue cell in the caudal fin, its changes of form at intervals for a period of twenty-four minutes being accurately depicted with the aid of the camera lucida. It is impossible to both watch and draw all the changes in form occurring during this period, but sufficient are shown to demonstrate very active amœboid motion. By observing these cells in relation to a relatively fixed object, *e. g.*, a chromatophore, or a joint in an adjacent fin-ray, I have been able to detect in them considerable locomotion. I find that, unlike the spindle cells, the stellate cells appear to travel equally well in all directions. Neither following their locomotion nor in trail of a retracted process do I find any indication of secreted granules, nor of anything other than the most delicate, preformed fibers. One would suspect that if new fibers were being formed by a process of secretion they would appear within twenty-four minutes after such a cell or its process had occupied a given position in which fiber should reasonably be expected to be formed. Though I have repeated the experiment many times I have failed to observe such evidence of secretion, and these results do not appear to be consistent with a theory of direct secretion of fibers by the primitive connective tissue cells.

The intracellular theories of the origin of connective tissue fibers may be divided into two classes, those in which the fibers are presumed to arise in the peripheral portion of the cell, as proposed by Lwoff (1889), or in a modified peripheral portion of the cellular or syncytial protoplasm, ectoplasm or exoplasm, as proposed by Hansen (1899) and afterwards by Mall (1902), and those in which the fibers are presumed to have a direct intracellular origin as observed by Spuler (1896) and later by Livini (1909).

The "circumcellular" origin of fibers proposed by Lwoff, since it must take into account the separation of fibers from the cells as well as their origin from intracellular granules, does not fundamentally differ from the later ectoplasmic modifications of Hansen and Mall. These later authors, basing their investigation on "fixed" material, presuppose a constant but gradual recession of the endoplasmic area with coincident changes in

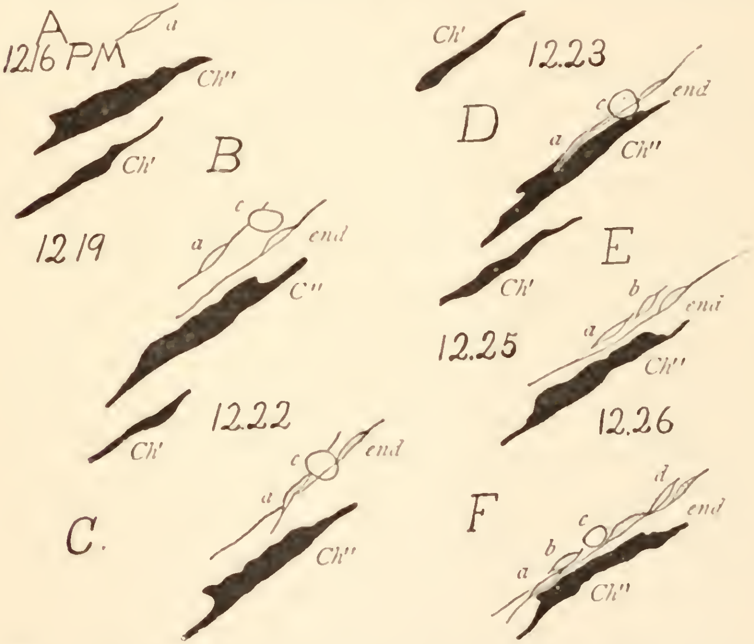


FIG. 2. A record of the locomotion of a spindle connective tissue cell, *a*, for a period of 10 minutes, adjacent chromatophores bordering on the fin-rays on either side being taken as the relatively fixed points. The cell was found to have covered a distance of about 50μ, a rate of 1μ in every 12 seconds. *a–d*, connective tissue cells; *c*, a round cell, the others spindle shaped; *Ch′*, *Ch″*, two chromatophores; *end*, endothelium of an afferent blood-vessel.

the exoplasm in order to account for the transference of fibers from a position within the cell, in the ectoplasm, to an extracellular position. This hypothesis is entirely harmonious with the appearances to be observed in "fixed" tissue with the methods used. However, it is open to two objections, viz., the observations of Spuler, confirmed in a casual and not wholly satisfactory way by Livini, that fibers actually lie within the cells, and not always at the periphery, as Hansen's and Mall's theories presuppose, but even in direct contact with the nucleus; and, secondly, if the cells are in active amœboid motion, with locomotion as well, as is certainly the case in the fins of the embryo *Fundulus*, then one can hardly conceive that the more or less fixed syncytium described by Mall, whose observations can be readily verified by any of the usual histological methods applied to the tissue used, is sufficiently stationary to permit the retraction or shrinkage of endoplasm, thus leaving the endoplasmic fibrils outside the bounds of the cellular protoplasm, for according to Mall's theory true cells are lost, being replaced by nucleated endoplasmic areas anastomosing with their neighbors to form a continuous syncytial net.

I have many times observed the fins of *Fundulus* embryos between the lengths of 4.5 mm. (at the time of hatching) and 25 mm., the pectoral and caudal fins being usually selected, and in no single instance have I observed either a stellate or a spindle cell which did not exhibit changes of form and locomotion, the latter being sometimes very limited, at other times, as shown in Fig. 2, quite extensive. Hence the ultimate theory of connective tissue histogenesis, it would seem, must take into account the relative activity of the mesodermic cells. So far as I appreciate them, none of the theories thus far advanced fully meet all of the observed conditions. Further studies of both living and "fixed" tissue may further elucidate the true conditions involved in the histogenic process.

The fins of living fish embryos offer for the development of connective tissue a most desirable subject for study, for in the subcutaneous tissue between the dermal fin-rays one finds a definite connective tissue area, in which, with the exception of the somewhat related chromatophores, there is no other structure to confuse the picture. This area is bordered on either side by the dermal fin-ray, beneath which an efferent blood-vessel descends, and alongside of which the return or afferent vessel ascends the fin; it is covered only by the pavement cells of the dermal epithelium, whose outlines are sharply defined, lying at a more superficial focal plane than the connective tissue. The chromatophores are easily recognized both by their color, black to yellow, and by their peculiar branching form, so that even those relatively or almost entirely devoid of color can be easily differentiated from the smaller colorless connective tissue cells. Moreover, the chromatophores are prone to lie in immediate relation to the fin-rays and to the blood-vessels.

In conclusion I desire to emphasize the opinion that in the study of histogenesis of connective tissue we must take into consideration the conditions surrounding the living cells and draw conclusions from "fixed" tissue only in the light of our knowledge of the living.

To furnish conclusive results living cells must be observed under normal conditions and not merely under those most unusual, or even pathological, conditions which surround the observation of growing tissues either in the mesentery studied by Flemming and others, open to the criticism of the presence of inflammatory changes, or in the tissue cultures by the more recent method of Harrison, Carrel and Burrows, which is surrounded by the necessity for the interpretation of results arising under entirely new surroundings and conditions as yet but little understood. Both in the mesentery and in tissue cultures movements of the connective tissue cells and even locomotion have been observed. I now add to these former results the record of the observation of motion and locomotion in cells under wholly normal conditions, in animals which underwent no operation other than in some instances the administration of chloretone, and which remained alive, active and normal for some time subsequent to the period of observation. For example, the particular fish which furnished the subject for Fig. 1 was returned to sea water and remained actively swimming for two days; he could have been kept much longer.

For the opportunity of pursuing this study I am indebted to the Marine Biological Laboratory at Woods Hole, Mass.

THE METHOD OF CELL DIVISION IN MONIEZIA.

C. M. CHILD.

Since the appearance of Richards's paper on the method of cell division in *Moniezia* (Richards, '11) Dr. Richards, in answer to a request of mine, has very kindly permitted me to examine some of his slides of early stages in the development of the ovary and of cleavage stages, with the privilege of publishing the results. The present paper consists of a brief statement of the results of my examination of these slides, together with some consideration of recent criticisms of my observations on amitosis. I take this opportunity of acknowledging my indebtedness to Dr. Richards and also of acknowledging an error of my own which though it does not directly concern the chief point at issue certainly requires correction. Dr. Richards is undoubtedly correct in his statement that the early cleavages in *Moniezia* are mitotic, either entirely, or to a much greater degree than my earlier statements would indicate. With this acknowledgment of my error the chief point of disagreement between Richards's positive observations, so far as they go, and my own is removed. The difference between us seems to me to rest now on Richards's failure to recognize, or to interpret as I have done, what the material actually shows.

The whole problem of amitosis seems to me somewhat unprofitable at present as a subject for investigation on a purely observational basis. Experimentation is necessary before we can reach any certain conclusions as to the significance of either mitosis or amitosis in the life of the cell. Moreover, cytological thought is at present dominated by morphological hypotheses, which are themselves based on direct observation rather than on experiment. So long as this remains the case all observations which do not accord with the current hypotheses will be explained away by one course of reasoning or another and brought into agreement with these hypotheses. So long then as our conclusions are based simply upon observation in fixed material and without any attempt to control conditions in that material there will always be room for endless discussion and controversy. It seems to me highly improbable that cytology will make any very great real advances in this direction. But when cytologists shall seriously devote their energies to the problem of the physiology of the cell, a field of investigation that has been entered only here and there, and to the control of cellular phenomena, then I believe we shall see real progress, together with the consignment to oblivion of some of the hypotheses now most fondly cherished.

As regards *Moniezia*, however, I have at present nothing to offer except further observations, or rather my own interpretation of what I have seen in Richards's material as against his interpretation of what he has seen. Beyond the present paper I do not propose to enter into any further consideration of the problem of amitosis until I can do so on a different basis, as I hope to be able to do at some time in the future.

Thus far I have only attempted to defend my own observations and conclusions against what seemed to me unmerited criticism and skepticism based on insufficient data. Richards's paper on *Moniezia* is, however, a real reinvestigation of the subject and as such I can only welcome it and accord it the consideration which is due to any piece of scientific work, although I am compelled to criticize it in various points. The present paper includes a statement of the results of my observations on Richards's slides, together with some discussion of his arguments and conclusions and brief reference to criticisms offered by other authors and to the validity of cytological theory.

The slides contain exactly the same pictures as my own. There is no question here of differences of fixation or of staining. If amitosis occurs in my material then it occurs in Richards's in the same regions of the body and the same stages of development.

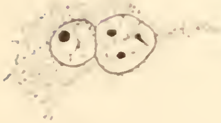
I. The Primordia of the Ovaries and Ducts.

Figs. 1–8 are camera drawings from the primordia of the ovaries in Richards's slides. They were drawn from several slides with different methods of fixation and staining. In the figures I have attempted to reproduce what I have seen as exactly as possible, though this is a somewhat difficult matter as regards the extranuclear phenomena. Godlewski ('09) has recently objected to my earlier figures on the ground that they were too highly schematized. This objection seems to me to be based largely on unfamiliarity with the material. In the early stages of the gonad visible cytoplasmic boundaries are often entirely lacking and the question as to whether cytoplasmic division follows nuclear division has no point, since it is quite impossible in most cases to distinguish cells. In later stages the germ cells do become distinct, but in those stages amitosis does not occur or occurs but rarely. In my earlier work I attempted to reproduce the essential features of the nuclei as exactly as possible. The only schematization consisted in not attempting to indicate an intranuclear achromatic structure except where such a structure was clearly visible and in leaving out various extranuclear details which did not bear upon the problem under consideration. In many of the nuclei the karyosome alone is stained and the rest of the nucleus appears almost or quite homogeneous; in other cases a more or less distinct structure of strands or an apparent reticulum does appear. In cases where I could not see such a structure distinctly I did not attempt to represent anything in the nucleus except the karyosome. It seems at least probable that the apparent achromatic nuclear structure is in many cases simply an artifact due to the action of the fixative. In the figures of the present paper I have represented a vague structure in all nuclei, but for most of the nuclei this must be regarded as little more than an attempt to show that something is there.

Richards's statement (p. 135) that the parenchyma is not a syncytium seems to me to require qualification. It may well be that it is not a continuous syncytium throughout the whole proglottid in later stages, but there cannot be the slightest doubt that several or many nuclei are often found imbedded in a more or less continuous mass of cytoplasm. This is true of the early stages of the gonads as well as of other parts. Where the nuclei are far apart they are usually, as Richards states, surrounded by more or less definite and distinct cytoplasmic masses, but this is certainly not true in cases where they lie close together.

Fig. 1 shows two nuclei in close contact, with the surfaces of contact flattened. If each nucleus surrounds itself with cytoplasm it is difficult to understand how a condition like Fig. 1 can arise except by division. Moreover, this is not the usual result of mitotic division in *Moniezia*, nuclei which arise by mitosis are separated by an appreciable distance when they form and are usually of equal size, which the two nuclei in the figure are not.

Fig. 2 shows a case which I am unable to interpret except as a direct division. The most careful focusing shows that the nuclei are in the same plane and that the membrane separating them is not a strand of "linin" but a real membrane. Moreover, this membrane is apparently thinner and more delicate than other portions of the nuclear membrane.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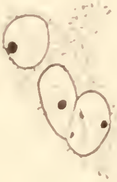
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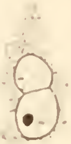
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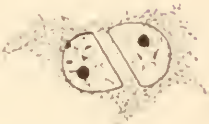
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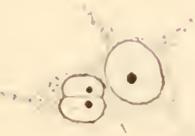
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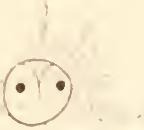
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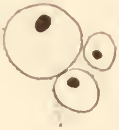
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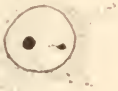
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In Fig. 3 two nuclei are shown which seem to be just completing division, much as in Fig. 1.

Fig. 4 shows two very distinctly hemispherical nuclei with flattened surfaces parallel and separated by a small amount of cytoplasm which stains less deeply than other parts. They appear as if division had recently occurred and cytoplasm had begun to form on the adjoining surfaces. If it were not for certain preconceived ideas concerning the method of cell division, I think no one would hesitate to regard such a case as a very strong indication—I do not say proof—of direct division.

In Fig. 5 the apparent division of a small nucleus is shown. The membrane between the two parts is more delicate than the rest. The position of the two "nucleoli" is important. They are close to the separating membrane and directly opposite each other. This position is of very frequent occurrence in such cases, far too frequent I believe, to be the result of chance. It suggests very strongly that division of the nucleoli may occur in many cases.

Fig. 6 is a case of apparent "endogenous" division. Richards (p. 14) was unable to find anything of this kind in the material. I found a number of such cases in his slides.

Figs. 7 and 8 show cases in which the two halves of a nucleus are slightly different in color, a phenomenon of not infrequent occurrence, which Richards also failed to observe. I do not know, of course, that such a condition indicates division, but I regard it as highly suggestive in that it certainly indicates some difference in condition in the two parts of the nuclei and a certain degree of independence of each other.

Figures of this sort might be multiplied indefinitely from Richards's slides, but there is no reason for giving others here. My chief object is to show that exactly the same pictures appear in his material as in my own. I am perfectly well aware that none of these figures and likewise none of my earlier figures constitute a real demonstration of the occurrence of amitosis, for such a demonstration is impossible in fixed material.

Numerous similar pictures were found in the early stages of the genital ducts, the vitellaria and the parenchyma during the stages when increase in the number of nuclei is occurring in these regions. There is, however, no reason for giving figures from these organs, for on the basis of current cytological theory the point of chief importance is whether amitosis occurs in the germ cells. If its occurrence is admitted for these cells, most cytologists would, I think, be willing to admit at once that similar phenomena in somatic cells doubtless indicate its occurrence there.

We may now consider the value of such pictures as evidence and the force of some of the criticisms made by Richards and others.

As regards the first point, I have observed pictures of this kind only in regions where the nuclei are increasing in number. Godlewski ('09) has suggested that I may have mistaken nuclear fusions for divisions. It would be strange indeed if nuclear fusion should occur only or chiefly in regions where the nuclei are undoubtedly increasing in number in some way.

Boveri's suggestion ('09) that the two nuclei in a cell represent the paternal and maternal chromatin refers primarily to the amphibian cells and perhaps he himself would not regard it as adequate to account for the appearances in *Moniezia*. Certainly there is no evidence of the separation of the two chromatins in mitotic division in *Moniezia*, even in cleavage. As regards the amphibian cells which I figured in an earlier paper (Child, '07*c*, Figs. 10 and 11) it should be repeated that the stages studied were not cleavage stages, but mostly larval stages, in which I could find no evidence, and I took especial care concerning this point, of a separation of paternal and maternal chromatin in mitotic division. I do not believe that this suggestion will account for the phenomena even in *Amblystoma* and it certainly will not in *Moniezia*.

As regards the possible increase of the nuclei by migration in particular regions, *e. g.*, the primordia of the gonads, Richards himself admits (p. 130) that the evidence for migration is not strong. Moreover, even if migration does occur in the formation of the gonads, there must nevertheless be a considerable primary increase in the parenchymal nuclei before the gonads are formed. Richards (pp. 138–9) finds the actual increase in parenchymal nuclei to be about equal to the increase in volume of the proglottids. Such an increase is, I believe, entirely inadequate to account on the basis of migration for the very large number of nuclei which appear in gonad formation.

Concerning the occurrence of mitosis Richards's actual observations are essentially identical with my own; he finds it but rarely, in fact he has not figured anywhere a case showing so many mitoses as my Fig. 8 (Child, '07*a*), in which seven cases of mitosis are shown. His argument concerning the ratio of resting to dividing cells in sections (pp. 139-40) concerns matters of which we really know almost nothing one way or the other; moreover, I am fully convinced that in many cases besides *Moniezia* where the infrequently observed mitoses have been supposed to account for all nuclear increase, numerous amitotic divisions actually occur. But besides all this, my conclusions concerning *Moniezia* are not based primarily on the frequency or infrequency of mitosis, but upon the actual observation of what I cannot regard as anything but amitosis. The infrequency or apparent absence of mitosis in many regions of *Moniezia* was what first led me to undertake a further investigation of the material and the remarkable infrequency of mitosis according to all observers in the neck region, the developing gonads and the genital ducts still seems to me to be highly suggestive, especially when we add to this fact the very frequent occurrence of what seems by all criteria at present available to be direct division.

Concerning periodicity in mitosis (Richards, '11, p. 140) the only positive observation which suggests anything of the sort is mine, which was cited in an earlier paper (Child, '10). This was a case of scolex, neck region and a few proglottids in which most of the nuclei were dividing mitotically. I have no means of knowing whether this is a normal occurrence, but if it is, it seems probable that this specimen is a young scolex, for the nuclei throughout the scolex are dividing as well as the others and these nuclei certainly do not take part in the formation of proglottids in later stages. I have considerable evidence for various forms which seems to me to indicate that many organisms or parts may start a period of nuclear multiplication by mitosis, while in later stages amitosis becomes increasingly frequent and if this scolex is normal it may be a case of that kind. It is quite possible, however, that it may be the result of certain special conditions of which we know nothing and not a normal stage of development in *Moniezia* at all.

It is certainly most strange, if mitosis occurs periodically or in waves as Richards suggests, that neither Richards nor Young nor I—except in the case above mentioned—have happened to observe any of these waves of mitosis in the numerous animals that we have sectioned. All the evidence which we have obtained indicates a remarkable infrequency and a scattered occurrence of mitosis.

But the argument based on mitosis is not direct evidence for or against the occurrence of amitosis. When we find the pictures which I have figured occurring frequently and confined to regions in which the nuclei are known positively to be increasing in number in some way, the direct evidence for the occurrence of amitosis seems to me to have far greater value than any indirect evidence based on the frequency or infrequency of mitosis.

On pages 149–50 Richards argues that in the primordium of the ovary and ducts the region of nuclear increase is at the periphery and states that it is here that mitosis is chiefly found. With the latter statement I agree, but I find it difficult to understand the grounds for his conclusion that nuclear increase is confined to the periphery. Certainly the increase in the number of nuclei at the periphery is not very great, while in the central region of the primordium the nuclei are very numerous, crowded close together. Moreover, these central nuclei are in early stages very much smaller than the peripheral. Unless there has been extensive migration from the periphery toward the center it is impossible to account for the great number of nuclei there except by proliferation. Richards has failed to find amitosis in this region, but I have found numerous cases of what I regard as amitosis in his slides. He maintains that the central nuclei are beginning differentiation (p. 150). I have been unable to observe any visible differentiation in this region at these stages and besides these crowded nuclei are on the average much smaller than any nuclei of the whole primordium after the visible differentiation has begun. In later stages, when, as I believe, proliferation becomes less rapid, these nuclei gradually enlarge again and become more widely separated and then visible differentiation begins. For these reasons I am unable to agree with

Richards that the region of greatest proliferation is the periphery of the primordium. Such a conclusion fails, it seems to me, to take account of all the observed facts. The great number, the very small size, the crowded condition of the nuclei, the relatively small amount or cytoplasm and the very frequent occurrence of apparent amitoses force me to maintain my original position, that the central region of the primordium is a region of more rapid proliferation than the peripheral. In connection with this point I have evidence from various species that in regions of more rapid proliferation amitosis is more frequent, while in regions of slower proliferation mitosis may be the chief or only method of division.

On p. 150 Richards states that "the method of cell multiplication in the female sex ducts of *Moniezia* cannot at the present time be positively stated." Here evidently the author has lost faith to some extent in his own argument, for the conditions in the ducts are not essentially different, so far as observation shows, from those in the primordia of the gonads themselves.

Of the possibility of observational error (Richards, '11, pp. 140, 141, etc.) no one can be more keenly aware than myself. I can only state again that I believe that I have used every precaution possible in direct observation of fixed material. I have often spent hours on a single case with the most careful focusing, changes of illumination with the aid of diaphragm and mirror and the alternating use of artificial light and sky light with and without color screen. Until Richards can show that he has taken at least equal precautions before concluding that direct division is absent, he lays himself open to the charge of error or superficial observation.

As regards the difficulty of observation in the small and often crowded nuclei of cestodes, a point which Richards emphasized in his earlier paper (Richards, '09), it is of course true that the nuclei are small and often crowded, but if one devotes sufficient time and care to the matter it is possible to find nuclei which show apparent direct division with almost diagrammatic clearness and I have seen many such, both in his material and in my own. In such cases I have not been able to convince myself by any means which I could devise that the appearance of direct division was a deception or an artifact, or that it lay within the limits of observational error, as Richards asserts for all apparent cases of the sort observed by him.

It would be of interest to know how far Richards is familiar with the appearance of amitosis in forms and tissues where it is generally admitted to occur. I have spent considerable time familiarizing myself with such material and I find many cases of apparently dividing nuclei in *Moniezia* which seem to me to be identical in appearance with cases of division in such tissues.

And finally, Richards admits finding numerous "paired nuclei" and nuclei between which no layer of cytoplasm could be distinguished; it is of interest incidentally to note that his statements on this point concerning the ducts and vitellaria are much less cautious than those concerning the ovarian primordium itself. And although both he and I have observed mitosis in the ducts, he is willing to admit concerning them that there is "no certain evidence for amitosis and that for mitosis is, perhaps, insufficient to account for the growth which has taken place" (p. 150). And concerning the vitellaria he says, "if mitosis is not clearly proved as the sufficient cause of cell multiplication, amitosis is certainly less so." Concerning the ovarium primordium his statements are much more guarded. Here the influence of cytological hypotheses appears. Most authors are willing to admit the occurrence of amitosis in somatic cells, though they would regard as rank heresy the assertion that it occurs in the germ cells. Certainly *Moniezia* affords no basis for such a distinction and as regards many other forms we have not the slightest evidence that the germ cells in early stages are different in any way from somatic cells. The most that observation can tell us on this point is that in some forms they appear early, in others late.

As to what constitutes evidence for amitosis Richards does not seem to be entirely clear. As regards the vitellaria he says "but for amitosis there is only negative evidence" (p. 147), yet in the same paragraph he admits that "the arrangement of nuclei in pairs is, perhaps, more in evidence here and indented nuclei are somewhat more numerous." These observed facts represent conditions which we should expect to find present if amitosis occurred and therefore constitute not negative but positive evidence of considerable value. They do not, of course, constitute a demonstration, but, as I have pointed out above, an actual demonstration or proof is impossible in fixed material. To my own mind, all the facts concerning which Richards and I agree, together with the cases which I regard as nuclear division, but which Richards has failed to find, constitute a practical, though not a logical demonstration of the occurrence of amitosis in *Moniezia*. On p. 127 Richards asks "whether the failure to find evidence of a certain process, using proper methods and exercising due diligence, is not positive evidence of the lack of that process?" Such a question can of course only be answered in the affirmative, but this does not constitute a refutation of my statement that Richards had presented only negative evidence for the occurrence of amitosis; it is of course merely the same statement in other words. And we are all aware that positive evidence of the absence of something must be of the strongest character before it can be regarded as refuting direct positive evidence of the presence of the same thing. Richards is unable to show with anything approaching certainty that mitosis is the only method of cell division in the ovarian primordium. I have presented positive evidence that amitosis occurs in addition to mitosis, though my evidence does not amount to an actual demonstration since that is impossible. I believe, however, that the evidence for the occurrence of amitosis in the ovarian primordia of *Moniezia* is almost if not quite as conclusive as that for its occurrence anywhere else in other species.

II. The Cleavage Stages.

As regards the cleavage of *Moniezia*, my examination of Dr. Richards's slides enbles me, as mentioned above, to correct the error into which I fell in maintaining that "cases of mitosis are rarely seen after the first cleavage, but amitosis is of frequent occurrence" (Child, '07*b*). Richards's observations as stated in his paper led me to believe that I must have been in error on this point and the examination of his slides removed any doubt which might have remained.

There is no question but that the earlier cleavages beyond the first were almost entirely absent from the proglottids on which most of my study of cleavage was made and that my Figs. 21–26 (Child, '07*b*) are in reality sections of considerably later cleavage stages than I had supposed.

The passage of the eggs into the uterus in *Moniezia* undoubtedly occurs periodically and as the eggs are fertilized in the course of this passage it follows that in any single proglottid containing cleavage stages not all the stages will be present. In the proglottids on which I based my study of cleavage it happened that first cleavages were numerous, *i. e.*, a period of passage of eggs from the ovary to the uterus had occurred recently. But as a reexamination of these slides after my study of Richards's material, shows, the stages between the first and occasional second cleavages and much later stages were almost entirely absent. My figures of supposed early cleavages are in reality sections of eggs containing a considerable number of nuclei, but the plane of section happens to pass so as to show only or chiefly the large nuclei. That I could mistake these for early cleavages was due to the fact that I was much more interested in the condition of the nuclei than in the course of cleavage, and my observations on the latter point were only incidental and so led to a wrong conclusion. Thus my error on this point is explained, but I do not offer the explanation as an excuse.

Furthermore, Richards is entirely correct in his assertion that during the earlier cleavages the blastomeres possess distinct boundaries and the egg is not a syncytial mass.

On the other hand my examination of Richards's material has enabled me to discover what seems to me a very important point that he has failed to note. In his material the early cleavages are, as he says, mitotic and the blastomeres distinct. The cytoplasm in these stages stains rather deeply and uniformly and is not vacuolated to any great extent. At certain stages, however, which may vary in different eggs to some extent, but at which the egg consists of a considerable number of blastomeres it undergoes a marked change in appearance. The cytoplasm loses its tingibility to a large extent and becomes highly vacuolated and the distinct boundaries of the blastomeres disappear. Apparently these boundaries disappear gradually, for in many cases some blastomeres remain more or less distinct while others have completely disappeared and in still other cases the whole mass of the egg appears to be a syncytium. In my earlier studies of the cleavage I interpreted these stages as stages in the appearance rather than the disappearance of the cell membranes.

In these and later stages I find, both in Richards's material and in my own, the most striking evidence for the occurrence of amitosis that I have seen anywhere in *Moniezia*. Here the nuclei are large and they are not crowded, consequently they can be studied with less difficulty and a larger number of good cases can be readily found.

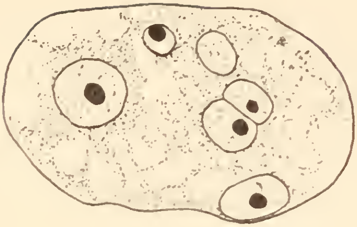
I give here a few figures drawn from Richards's slides to supplement my earlier figures.

In Fig. 9 five nuclei, one of them "double," are shown. No cell boundaries are visible and the cytoplasm is highly vacuolated and stains only slightly The two parts of the double nucleus are in immediate contact and the relation of the nucleoli to the dividing membrane and to each other which was mentioned above is seen.

Fig. 10 shows four nuclei, one of which appears to be dividing or to have recently divided. The two parts of this nucleus are of different size and are closely apposed.

Fig. 11 is an interesting case: the blastomere containing the double nucleus still retains its dense unvacuolated cytoplasm and to some extent its distinctness, though no actual cell membrane can be seen. The two parts of the nucleus which it contains are in immediate contact and flattened against each other. It is difficult to understand how they could attain such a position as the result of mitotic cleavage like that of the earlier stages. Moreover, in those earlier stages neither Richards nor I have found any evidence of the separation of paternal and maternal chromatin.

In Fig. 12 is shown a case of apparent division which seemed to me, as I examined it, particularly convincing. Here the two parts of the nucleus are of different size and a new nuclear membrane appears to be in process of formation between them. Its formation apparently began on one side and on that side the



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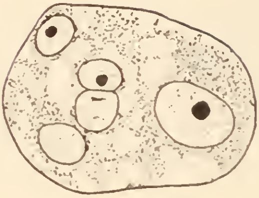

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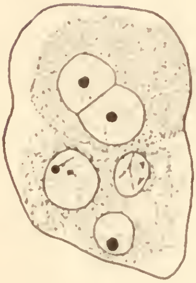

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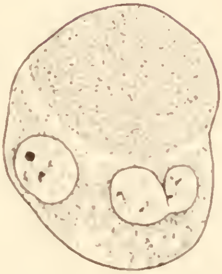

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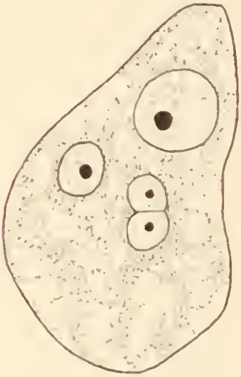

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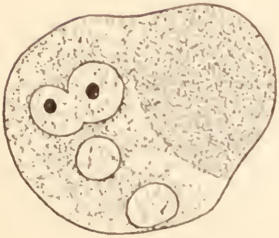

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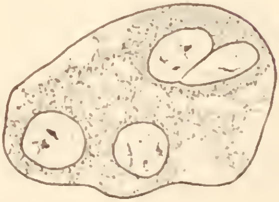

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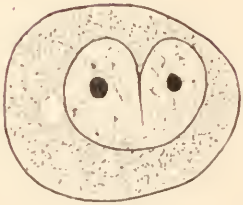

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nuclei have separated, while on the other side it is still very delicate and the nuclei are still closely connected. The dense mass in the upper part of the figure is the cytoplasm of a large blastomere which has not yet undergone the change in appearance; its nucleus is in another section.

Fig. 13 shows a section without any cell boundaries and with vacuolated cytoplasm in which one small nucleus is apparently dividing.

In Fig. 14 a constricted nucleus is shown. The cytoplasm of a large dense blastomere is also seen in the figure.

Fig. 15 shows another case which is to me very convincing. The double nucleus is in a mass of cytoplasm which is denser than the rest and still rather distinctly marked off from it. Evidently this represents a blastomere. Here, as in Fig. 12, the division of the nucleus apparently began from one side, for the membrane is much more distinct on the side where separation is most advanced and becomes more and more delicate toward the other side.

Fig. 16 represents a case of almost diagrammatic clearness in which a large nucleus is apparently dividing from one side. Other nuclei are in other sections of the egg.

Figures of this sort might be multiplied indefinitely from Richards's material as well as from my own. There is, I think, but little chance of observational error here, for the nuclei are relatively large and not crowded and in many cases almost diagrammatically distinct. If pictures of this sort are of any value as a criterion of the occurrence of amitosis, then the evidence is certainly very strong for these cleavage stages.

One question, however, must be raised here, viz., whether these are normal stages in development or degenerating embryos. To settle this point absolutely the study of later stages is necessary and thus far I have not been fortunate enough to obtain such stages. But in many proglottids practically every embryo shows this syncytial condition and if this indicates degeneration, then all, or almost all, the embryos of those proglottids are degenerating. It seems probable therefore that we have to do here with normal stages in the development.

III. Conclusion.

My examination of Richards's material has only confirmed me in my conclusion that direct division plays an important part in the developmental cycle of *Moniezia*, in the germ cells as well as in the soma. I believe I have at least shown clearly enough that this material contains something besides negative evidence for amitosis.

We are, I think, not yet acquainted with the nucleus as a dynamic system. We know something of it morphologically and almost all our hypotheses and theories concerning it are based on the morphological data obtained from fixed material. But even when we consider only the results of direct observation it is difficult to understand how cytologists can continue as many do to ignore or minimize the importance of the rapidly increasing number of observations on the occurrence of direct division. Certainly the work of recent years on protozoa, to mention only this group, has brought to light many apparent facts which are not in agreement with current cytological theory. Where there is so much smoke it would seem that there must be some fire.

Moreover, when we regard the nucleus as a dynamic system rather than a morphological structure the necessity for assuming the individuality or continuity of the chromosomes disappears entirely. The reappearance of a definite and constant number of chromosomes in successive cell generations is essentially the same problem as the reappearance of five fingers on the hand in successive generations of man, or any other case of the inheritance of organs or parts in definite constant number yet we do not regard it as necessary to assume that these fingers or parts maintain their individuality at all stages of the life cycle. Instead of being the basis of heredity the chromosome is itself a problem in heredity. The theory of so-called chromosome individuality is a wholly unnecessary hypothesis and a heavy burden for cytology to carry, since it is not in accord with the facts and requires supplementary hypotheses at every point.

There is every reason to believe that the nucleus, like other dynamic systems, will behave differently under different internal and external conditions. What conceivable reason is there to doubt that it can under certain conditions undergo fission or fragmentation and still retain the capacity to become a "whole" again? We know that the capacity for regulation is a very general characteristic of living things: are we justified in asserting without the strongest experimental evidence that the nucleus is wholly without this capacity?

If it were not for the fact that it makes no essential difference for the phenomena of life, including those of heredity, whether the chromosomes are continuous individuals or not this hypothesis would doubtless have been abandoned long ago. But since it is in a sense outside the field of scientific investigation, at least so long as present methods of cell study are in vogue, the chromosome theory has developed into a wonderful inverted pyramid of hypotheses in the construction of which the chromosome appears as a veritable *deus ex machina*.

The great need of cytology is a substitution of the experimental for the descriptive method: more experimentation and control and less inference from observation, a dynamic rather than a static point of view, rigorous proof rather than loose speculation; with such changes we may hope to gain some knowledge of the cell. The disadvantages of the present method are sufficiently manifest in the present paper.

Hull Zoölogical Laboratory,
University of Chicago,
July, 1911.

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DROSOPHILA AMPELOPHILA LOEW BRED IN THE DARK FOR SIXTY-NINE GENERATIONS.[1]

FERNANDUS PAYNE.

In a short note (BIOL. BULL., '10) I stated that I had been breeding *Drosophila* in the dark for forty-nine generations or more than two years; that the darkness had produced no visible effect either in the color of the body or in the structure of the eye; but that there seemed to be a difference in their reactions to light, the ones bred in the dark reacting more slowly than those bred in the light. The evidence on which this last statement was based was meager. It was obtained by placing a number of flies in a clean vial and then revolving it so as to bring first one end and then the other toward the light. In this way it was demonstrated that the flies bred in the dark were noticeably much slower in their reactions than those bred in the light. These observations as far as they went were correct, but as we shall see they were not extensive enough and hence led to an erroneous conception.

I have just finished an experiment in which each fly was tested individually. This experiment was based upon four series of flies, 1,000 in each series: first (series no. 1), those bred in the dark for sixty-nine generations; second (series no. 2), those bred in the dark for sixty-four generations and then placed in the light for six generations; third (series no. 3), those bred only in the light; and fourth (series no. 4), a new strain from series no. 3 bred in the dark for five generations.

The apparatus for testing each fly consisted of a Welsbach lamp, a heat screen three inches thick and a glass tube one inch inside diameter and nine and three eighths inches long. The apparatus was arranged as shown in the diagram (Fig. 1), the end of the tube toward the light being ten and three fourths inches from it. The glass tube was perfectly clean on the inside and wrapped with black cloth on the outside. The flies were introduced into this tube by means of a shell vial three and one half inches in length and large enough to slide over the end of the glass tube. The end and about one half inch of the side of this vial were also covered with black cloth so when the vial was slipped over the tube, the tube admitted no light except at the end toward the lamp. By means of a stop watch I obtained the time it took each fly to travel the distance of nine and three eighths inches toward the light. Sometimes the flies did not react and in such cases they were counted out at the end of one minute. Records of these flies were also kept. This limit was placed at one minute, as experience showed that if they did not react within this time they might not react for five or ten minutes, or even longer. Extreme care was used to have external conditions as nearly uniform as possible. The temperature of the room at the time of testing varied between 74 and 78 degrees Fahr., but I could not see that a variation within this range made any difference. All flies were changed to perfectly clean vials and allowed to stand from ten to thirty minutes before testing, so they might become accustomed to a clean surface such as the inner surface of the tube into which they were introduced. The flies bred in the dark were placed in the light during this ten to thirty minutes in order to overcome any temporary effect of the darkness. All flies were tested at approximately the same age, from six to twenty-four hours after hatching. They were fed on banana which was permitted to reach about the same degree of fermentation before using.

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[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 120.

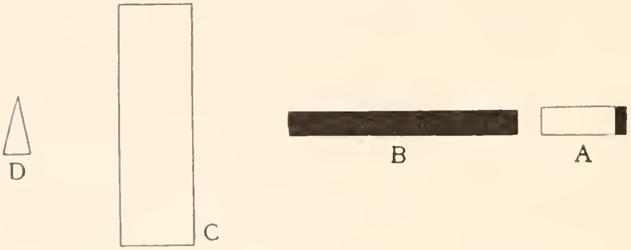

FIG. 1. Arrangement of the apparatus. *A*, the shell vial, three and one half inches in length; *B*, the glass tube, nine and three eighths inches long; *C*, the heat screen, three inches thick; *D*, the light, ten and three fourths inches from the end of the glass tube. The parts colored black were wrapped with black cloth.

With these precautions it was found that offspring of different parents within the same strain, those bred in the light as well as in the dark, vary considerably in their reactions. Further, this difference does not seem to be due to inheritance from the parents. To test this I selected five flies, each of which failed to pass through the tube in five minutes. The next day, I retested these same five flies and again they failed to react. This sluggishness was not due to poor development, as they looked strong and vigorous. These five flies were bred and their offspring tested. The average time of those which passed through the tube and the percentage which failed was approximately the same as for the series, indicating that something other than inheritance is the cause of this difference in reaction. I have taken two pairs, brothers and sisters, fed them on the same food, kept them side by side in the same room and yet their offspring showed a decided difference in their reactions to light. This fact has caused me a great deal of trouble and led to the erroneous conception stated in the previous note. In fact I can obtain the same result between the offspring of different parents in the same strain which I formerly stated was obtained by comparing flies bred in the dark with those bred in the light. Often this difference is so marked that it is noticeable before the flies are tested, those reacting least being more easily excited by handling. The only possible cause of this difference which I have to suggest is the food, as it may undergo different degrees of fermentation after it is placed in the vial. Another thing which leads me to believe that the food may be the underlying cause is that the last flies taken from any one vial are usually more sluggish than the first ones. Even with this difference in the reactions of the offspring of different parents, I believe the reactions of 1,000 individuals from many different parents gives a close approximation to the average for the strain.

The following table gives the average time of the flies in passing

1,000 Flies in Each Series.	Average Time of Flies which Passed through the Tube.	Percentage of Flies which Failed to Pass through the Tube in One Minute.
Series No. 1	13.9 seconds.	25.1 per cent.
Series No. 2	14.24 seconds.	23.6 per cent.
Series No. 3	17.62 seconds.	29.5 per cent.
Series No. 4	15.89 seconds.	23.2 per cent.

through the tube and the percentage which failed to pass through in one minute.

This table shows that the average time of the flies bred in the dark for sixty-nine generations (series no. 1) is least, 13.9 seconds, and the average of those bred only in the light (series no. 3) is greatest, 17.62 seconds. This is a difference of 2.72 seconds in favor of the flies bred in the dark. Likewise the percentage of flies which failed to pass through the tube in one minute is less (25.1 per cent.) in series no. 1 than in series no. 3 (29.5 per cent.). Series nos. 2 and 4 were run as controls and the average time is intermediate between the two extremes, while the percentage which failed to go through the tube is less than in series nos. 1 and 3. However, I do not believe the difference between any of the series great enough to be of any special significance and the only conclusion which can be drawn is that the darkness operating through a period of sixty-nine generations has produced no visible effect in the reactions of *Drosophila* to the light of a Welsbach burner. It should be pointed out, however, that the only cave condition present in the experiment is darkness; that it is possible and indeed probable that factors other than darkness (constant temperature and moisture) may play some part in the changes which have taken place in cave animals. If this be true the real test comes not in rearing animals in the darkness but in a true cave environment.

That the constancy of the environment may be a potent factor in the production of degeneration in cave animals is strengthened by the recent unpublished experiments of Tower. In a letter, August 2, 1911, Professor Tower makes the following statement:[1] "It has been my experience that there is hardly anything so injurious to breeding stock as a constant environment. It will produce degeneration, reduced activity, and not infrequently will result in actual elimination of the race." Also in the experiments of Calkins and others on protozoa, it seems that the constancy of the food supply and the constancy of the chemical make up of the medium in which they lived, brought about, at least a shorter cycle than occurs in a food supply and medium which are changed at intervals as shown by Woodruff's experiment (*Archiv für Protistenkunde*, '11).

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[1] I wish to thank Professor Tower for the permission to use this statement before it has appeared in print.

In caves conditions are practically uniform. Also in dark corners under stones and logs, conditions are more nearly uniform than in the open. Since it seems almost certain that forms which now inhabit caves once lived outside under stones and in dark corners and that they had varied in the direction of cave animals before they entered caves, is it not possible that the more nearly constant environment of the dark corners under stones and logs has started the degenerative changes which have been carried to their present condition in the more constant environment of the cave. To be sure all changes in cave animals are not degenerative. The tactile sense, for example, becomes highly developed, but no doubt a second causative factor enters here.

SOME PARASITES OF SIMULIUM LARVÆ AND THEIR EFFECTS ON THE DEVELOPMENT OF THE HOST.[1]

E. H. STRICKLAND,

CARNEGIE SCHOLAR IN ECONOMIC ENTOMOLOGY.

During the spring of the present year (1911) while studying entomology at the Bussey Institution of Harvard University, I made numerous collections of *Simulium* larvæ, which are extremely abundant in the neighboring streams, with the intention of studying the development of the imaginal discs which are unusually well defined in this genus of diptera. As, however, I found that a large percentage of these interesting larvæ were heavily parasitized by two very different organisms, namely a worm and a protozoön, I turned my attention rather more directly to these and their effects on their hosts, during the few weeks intervening between my first discovery of the parasites and the pupation of the insects.

Before giving any details I wish to take this opportunity to offer my sincere thanks to Professor Wheeler, who by his kind suggestions and advise enabled me to bring together the following facts, which, though very incomplete in form, do not appear to have been recorded before. My thanks are also due to Professor Johannsen for naming the species of *Simulium* larvæ and to Professor T. H. Montgomery who identified the worm parasite as a species of *Mermis*.

LIFE HISTORY AND STRUCTURE OF SIMULIUM LARVÆ.

A brief summary of the structure, and mode of life, of the *Simulium* larvæ may not be out of place here.

If during the months of March to May one examines the rocks or vegetation in any swiftly flowing stream in the neighborhood of Forest Hills, Mass., especially where its bed causes a small cascade, one will, in all probability, observe a large, dark, gelatinous mass where the current is swiftest and the water

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[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 45.)

shallowest. On closer examination the mass will be seen to consist of numerous curiously shaped larvæ standing upright on the rock, to which the hind end of the abdomen is firmly attached. These larvæ, the largest known North American species of which measure when mature some 12 mm., have the following characters: The soft-skinned body is more or less cylindrical, though the posterior third is somewhat swollen. The segments are poorly defined but the first three behind the head, namely, the thoracic segments, are usually distinctly marked off from the following abdominal segments.

The prothoracic segment bears a single leg (Plate I., Fig. 1) which is apparently two-jointed; the distal joint is small and retractile and terminates in a sucker which is armed with numerous hooks. No other segment bears any trace of legs, with the exception of the apical abdominal segment where the pair of anal prolegs of some other larvæ is represented by a powerful, armed disk-like sucker, by means of which the larva firmly attaches itself to rocks or vegetation when at rest. The skin is usually of a greenish gray color in immature larvæ, but gives place to a reddish brown as they mature.

The head is sub-cylindrical and is usually of a darker color than the rest of the body. Its chitinous integument is much denser and less elastic than that covering the remainder of the body. This results, during growth, in an ecdysis of the head capsule alone, being necessary more frequently than that of the general cuticular covering of the body. The new head capsule exposed after such an ecdysis is perfectly pigmentless in *Simulium hirtipes*, though dark spots soon form on its vertex (Plate I., Fig. 2, *a*, *b* and *c*), followed by a gradual infuscation of the whole surface. Individuals were observed in which the almost black head capsule was half removed, exposing the new pigmentless capsule, entirely independently of the body cuticle, the ecdysis of which was never observed, except at pupation.

On either side of the head are two black eye spots and on the anterior border are two lateral fan-like organs borne on elongated pedicels, which are used by the larva in procuring food. These fans consist of numerous curved rakes (Plate I., Fig. 3) bearing on one side long stiff cilia which, when the fan is expanded, stretch from one rake to the next, thus forming a very fine strainer which allows water to pass through it, but retains any small organisms, such as the diatoms, on which the larva subsists. By means of a curious flicking motion these fans can be closed and their contents brushed into the mouth orifice. Here are situated two large glands (Plate IV., Figs. 9 and 10 (*d*)) on the dorsal side of the pharynx, whose function appears never to have been determined. They are covered with an apparently porous membrane which is clothed with short stout hairs. It would seem that they secrete some sticky material onto these hairs which removes the particles of food from the rakes when the latter are brought in contact with them.

It is usually stated that the fans are used to set up currents in the water and thus sweep food toward the mouth. My observations, however, lead me to believe that they act as a "strainer" and this is supported by the fact that, living as these larvæ do, in the swiftest currents, such movements would be useless.

The salivary glands are very large and secrete powerful silken threads which are used by the larvæ as anchor lines to hold them in an upright position no matter how strongly the current of water may flow.

Although the larvæ appear to be very sluggish, they can move about actively on the rocks by a looping motion similar to that of the geometrid larva. It is interesting to note the care with which these larvæ, when thus moving about, assure themselves that the adhesive disc of the prothoracic leg is firmly attached before relaxing the anal disc and *vice versâ*. Respiration is accomplished by means of retractile finger-like blood gills, normally three in number, which are situated on the dorsal surface of the last abdominal segment just above the anus. These gills, which can be distended at will by the insect, by means of blood pressure, are apparently inadequate for use in any but rapidly flowing water which contains plenty of oxygen, for if the larvæ be placed in a jar of still water very few will survive for more than eight hours, though life may be prolonged for two or three days by placing them in small numbers in Petri dishes containing only sufficient water to just cover them.

The form and size of the imaginal discs, or histoblasts, will here be described in detail as they are of especial interest in connection with the parasites.

The thoracic histoblasts are twelve in number and are very large and conspicuous in normal larvæ as they approach full growth (Plate I., Fig. 4).

The six discs to be found on each side of the thorax are the following:

1. Adult prothoracic leg histoblast—situated at the base of the larval prothoracic leg.

2. Adult mesothoracic leg histoblast—situated ventro-laterally on the mesothoracic segment.

3. Adult metathoracic leg histoblast—situated ventro-laterally on the metathoracic segment.

4. Adult wing histoblast—situated dorso-laterally on the mesothoracic segment This in the mature larva is by far the largest disc and it soon comes in contact with the mesothoracic leg disc.

5. Adult halteric histoblast This in the early stages is almost as large as the wing disc, but its growth and development are very slow and it soon disappears under the rapidly expanding wing disc.

6. Pupal respiratory tuft histoblast. This is situated on the prothorax and in its young stages has the appearance of being quite homologous with a prothoracic wing. It, however, soon begins to take on a definite form, and the comparatively stout tracheal tubes can be seen growing and lengthening beneath the transparent cuticle till they become coiled up as indicated in Plate I., Fig. 4.

It should be noted that this histoblast is not in the true sense of the word an "imaginal" disc since the respiratory filaments are exclusively pupal organs, and have no equivalent structure in any adult. Their similarity to a prothoracic wing destitute of the wing membrane is of interest. A few days before pupation this "pupal" histoblast begins to darken. Pigmentation commences at the apex of the folded tubes and slowly works backwards toward the base when the disc takes on the appearance shown in Plate III., Fig. 8*b*. Later, immediately prior to pupation, the cuticle over this disc ruptures and liberates the fully formed and now functioning filaments.

The growth of these various histoblasts causes the thorax to swell considerably, thus giving the body the appearance of being constricted in the middle.

Internally there is comparatively little tissue. The abdomen contains the alimentary tract, and the much elongated salivary glands which lie normally in a ventro-lateral position with regard to the alimentary tract. The sexual organs are small and not easily found even in serial sections. The remaining portions of the body cavity are filled with blood plasma in which is suspended a quantity of fat body, mainly collected near the apex of the abdomen and causing this region to become slightly swollen. As the larvæ mature this fatty tissue very materially increases, and when dissected out, is of a stringy nature.

METHODS.

Most of the larvæ were killed as soon as they were brought into the laboratory, but a few of the more heavily parasitized ones were kept alive in running water by covering the mouth of a jar with fine netting and introducing a piece of rubber pipe into the jar, through which tap water was run. In this manner specimens were kept alive for several days.

The following killing fluids were found to be the most satisfactory among several tried:

1. *Hot Water.*—Water was just brought to the boil when the larvæ were immersed and allowed to cool in the water. This method was most unsatisfactory from a histological point of view, but it had the advantage of leaving the skin as transparent as in its normal condition. It was also possible to dissect larvæ thus killed.

2. *Gilson's Fluid.*—This was used hot as described above and gave good results, but had the disadvantage of making staining with the hæmatoxylins difficult.

3. *Kahle's Fluid*, consisting of 30 parts water, 15 parts 96 per cent. alcohol, 6 parts formalin (40 per cent.), 1 part glacial acetic acid. This fluid has been recommended by W. Kahle (1908) and proved to be superior to Gilson's fluid both for

fixing and staining, and in the end was exclusively used. It was also used hot.

The chief advantage of both of these fluids was that, besides giving excellent histological preparation, they caused the histoblasts to turn milky white so that their position and form could be readily observed immediately after the specimens were killed.

Staining.—Heidenhain's iron hæmatoxylin combined with orange G was almost entirely used as it gave the best differentiation, though staining was rather slow. Replacing orange G with eosin accelerated staining but differentiation was less precise.

MERMIS SP. PARASITIZING SIMULIUM LARVÆ.

A number of larvæ were placed in a jar of water and left over night. The following morning I was surprised to see several white worms moving about at the bottom of the jar. It was evident that these had come out of the *Simulium* larvæ, so I went out to a ripple where the larvæ were particularly abundant and examined the colonies on several stones. I then noticed the large size of many individuals, and on examining these I found that many of them had a worm coiled up within the abdomen (Plate I., Fig. 5). When a quantity of material was brought into the laboratory it was found that these parasitized larvæ were much more sluggish than their healthy companions, which rapidly explored the jar in which they were confined, with their peculiar looping gait. The parasitized individuals, however, seemed much less concerned as to their new surroundings, and many of them were soon motionless, standing upright on the bottom and sides of the jar with their rakes expanded, ready to catch any food which might float their way. Here is a possible explanation of their larger size. Owing to their curious form of feeding it is evident that the more sluggish an individual is the more food it can obtain, and should it grow but a little larger than its companions, it will reach above their innumerable outstretched rakes, so that its food supply will be very materially increased.

It must not however be taken for granted that all parasitized larvæ were larger than their healthy companions, for some remained quite small, whereas many of the larger larvæ showed no signs of worm parasites. As a general rule, however, the largest larvæ were found to contain one or more worms coiled up within the abdomen. One would naturally expect this rule not to be very constant, if, as conjectured, it is simply due to a more sluggish temperament and increased appetite on the part of the parasitized larvæ.

A case of a *Mermis* parasitizing ants was described by Professor Wheeler ('07) and here also he noticed a great increase in size of the host due to a greatly increased appetite during the larval stage.

A number of the worms were dissected out and sent to Professor T. H. Montgomery who pronounced them to belong to an undetermined species of *Mermis.*

RETARDATION OF DEVELOPMENT OF THE HISTOBLASTS DUE TO MERMIS.

On making a closer examination of the parasitized larvæ a far more remarkable effect than that of increased size was noticed, for it was found that the presence of *Mermis* parasites, no matter in what numbers, has a direct effect on the development of the histoblasts. In a normal larva of about 10–10.5 mm. which is the maximum length, and is attained immediately prior to the blackening of the respiratory filament histoblasts, the latter are quite large and owing to their white color readily visible to the naked eye; especially when the larva has been killed in Kahle's or Gilson's fluid (Plate II., Fig. 6). If a parasitized larva of the same, or greater, size be examined no trace of the histoblasts can be discovered with the naked eye, and can only be detected with difficulty under a dissecting lens. Under the low power of a compound microscope, however, they are seen to be represented by small white traces of the organs which should at this time be far advanced in development (Plate II., Fig. 7). A close examination fails to reveal any differentiation of these retarded histoblasts into the component parts of the adult, or pupal, organ.

The parasitized larva rarely develops beyond this stage, though I have observed specimens which were turning reddish brown, and contracting slightly as a healthy larva would, shortly before maturation. In some cases, even, the rudimentary respiratory filament histoblast begins to darken in color.

I examined numerous specimens from the same stream at Forest Hills, and, with slight variations in intensity, they all showed this nearly complete suppression of the imaginal discs. Some weeks later I chanced to pass a small stream at Norwood, about seven miles from Forest Hills, and seeing *Simulium* larvæ present in small numbers, I took nine specimens from the rocks in order to see whether the species was identical with that common in our streams at Forest Hills. They proved to belong to the same species, namely, *Simulium hirtipes*, but I was much surprised to find that here also the *Mermis* parasite was much in evidence for of the nine specimens taken, six contained the worm. The effects however in these specimens were not so marked as in those found nearer home, for four of the six had turned brown and although the histoblasts were much smaller than is normally the case they were readily visible to the naked eye. Undoubtedly, however, maturation was impossible, for even should the larva manage to pupate it would soon die for want of oxygen, since the respiratory filaments were but half formed.

In a third locality, the Stonybrook Reservation near Forest Hills, a different species of larva was found. Professor Johannsen states that this larva is quite new to him and may be the undescribed larval stage of *S. bracteatum*, which occurs frequently in this State, but as I found only adults of *hirtipes* it was impossible to confirm this supposition. These larvæ were, however, also found to suffer from *Mermis* parasitism, but to a much less extent than those of *S. hirtipes*. The effects on the host were, however, as one would expect, identical with those on *hirtipes*, though the increased size was not so evident.

A large number of larvæ were measured, cut open and the worms removed. It is interesting here to note that even in fixed specimens where the fluid had caused the skin to become opaque, the presence or absence of worms could in all cases be determined with certainty by a glance at the histoblasts. The worm, it should be noted, did not cause the abdomen to swell up or become distorted to any appreciable degree.

A few selected results shown by the examination are appended:

	Length.	Color of Larva.	Histoblasts.	No of Worms.	Size of Worms.
1	10.75 mm.	Gray.	Minute.	12	Av. .75–1 cm.
2	11.50 mm.	Gray.	Minute.	1	2.75 cm.
3	11.0 mm.	Gray.	Minute.	1	3.00 cm.
4	11.0 mm.	Gray.	Minute.	3	1.3, 1.5, 1.5 cm.
5	10.5 mm.	Brownish.	Minute.	1	3.00 cm.
6	9.0 mm.	Gray.	Quite small.	3	.9, 1.3, 1.5 cm.
7	8.5 mm.	Brownish.	Large.	0	———
8	10.5 mm.	Gray.	Large.	0	———
9	8.0 mm.	Brownish.	Large.	0	———
10	7.0 mm.	Brownish.	Large.	0	———
11	8.5 mm.	Gray.	Large.	0	———
12	11.0 mm.	Gray.	Large.	0	———

From this table it will be seen that when there is but one worm in a host it attains a length of about 3 cm. during its parasitic life; that is, roughly about three times the length of the host itself, since the average length of a parasitized larva is 10.5–11 mm. It will also be seen that in one case as many as twelve worms were removed from a single host, and that in this case they all remained small, owing doubtless to insufficient food supply. In other instances, however, where several worms were present in one host it was noticed that one had attained almost to the normal size of 3 cm. whereas the remaining worm or worms were less than 1 cm. in length.

The larval measurements showed that parasitized larvæ average about 11 mm. (Plate III., Fig. 8*a*) whereas mature, and therefore somewhat contracted, larvæ, average some 8–8.5 mm. (Plate III., Fig. 8*b*), though a few are abnormally large.

Life History of the Worm.

As yet nothing is known of this except during the latter part of the life in its host. In all probability the eggs or young worms, are caught as they float down stream by the outspread larval rakes and are swept into the mouth, passing into the alimentary tract through the walls of which they bore their way till they enter the body cavity. It should however be noted that no scars or hypertrophy of the wall of the alimentary tract were noticed in several serial sections made of parasitized larvæ. The alternative hypothesis is that the larval worms bore into their hosts through the thin cuticular covering of the abdomen. That they should be present in the *Simulium* eggs is hardly possible, and this hypothesis can be with safety rejected.

Either of the former hypotheses offers a possible explanation for the variation in development of the histoblasts of parasitized larvæ taken from different streams, for in the case of those taken at Norwood where the discs were of a moderate size, the worms may not have entered the body cavity till the discs were somewhat developed. Then again where one worm has grown to nearly the normal size of 3 cm. whereas other worms in the same host have remained small, it is probable that the large worm entered the body cavity some time before the others.

Figure 9, Plate IV., which is a drawing of a median longitudinal section of a parasitized larva, shows the ventral position of the worm with regard to the alimentary tract, though it will be seen that the mesenteron has been somewhat pushed to one side by the coiled parasite. It will also be seen by comparing Fig. 9, Plate IV., with Fig. 11, Plate III., that the parasitized larva has much less fat body than that of a healthy individual at about the same stage of growth.

Another effect of the host is that apparently the sexual organs do not develop in parasitized larvæ. These are not easily seen in a healthy *Simulium* larva, as they are very small, but they can usually be found in a good series of sections; in sections of parasitized larvæ I have been entirely unable to locate them.

Otherwise the parasite appears to have no effect on the internal organs of the host, with the exception of displacing the spinning glands, the largest portions of which normally lie in the position later occupied by the parasite. Although they may be so displaced as to lie dorsad of the alimentary tract (Plate IV., Fig. 9, *b*) their functioning is in no way impaired, since these larvæ spin quite as many silken threads as do those which are uninfested.

The *Mermis* probably feeds directly on the blood plasma and fatty tissue of the host.

As the larva reaches maturity the contained worm becomes very restless, and if a living larva be placed under the low power of a microscope in a cell slide the movements of the worm can be easily observed. One thus watched for about half an hour was seen to explore the hypodermis of the host, evidently attempting to find a place of exit. The head was kept constantly moving over the internal surface of the abdominal hypodermis and was even sometimes thrust far into the thoracic region. The movements appeared to cause the host great pain, especially when the thoracic region was visited. Finally after the entire worm had been several times twisted round in the body the head was pressed against the junction of the third and fourth abdominal segments and a hole was quickly made through which the worm slowly emerged. The worm, measuring 3 cm., took in all 27 minutes to disengage itself after puncturing the skin of its host. At first the operation was very slow and the constant writhing and turning of the host impeded rather than aided the movements of the parasite. When about 50 mm. were exposed the head was twisted around the body and the remainder of the worm was more rapidly forced out, finally becoming detached from the host in a tightly knotted mass which soon straightened out. The larva meanwhile rapidly shrank, and in about half an hour was dead. The worm apparently can leave the abdomen at almost any point, though the thin junction between two segments is usually chosen. It is probable that the death of most parasitized larvæ is directly due to the escape of the worm, which in all observed cases occurred some time before maturity.

On raising leaves in the bed of a stream a little below a large colony of *Simulium* larvæ it was found that they had under them several of these white worms. This year most of the worms had escaped by the beginning of May, and have since been lost sight of, though while examining a stone microscopically for a second brood of *Simulium* eggs, on May 29, one or two minute worms were seen, which may have been young *Mermis*. No *Simulium* eggs could be observed. At a still later date, August 3, a full grown, though dead, *Mermis* was found under a stone in the bed of the stream.

PERCENTAGE OF LARVÆ INFESTED.

Several leaves covered with larvæ were taken from a stream in Forest Hills for the purpose of determining the percentage of parasitism.

These larvæ, which represented the species *Simulium hirtipes*, gave the following results: Number of larvæ present 174; number of parasitized larvæ 41. This means that in this particular locality about 23.5 per cent. of the larvæ would be unable to mature on account of the *Mermis* parasite.

The species living in the Stony Brook Reservation was found to be parasitized only to the extent of some 3–4 per cent. A few *S. hirtipes* were also present in the same stream and were parasitized only to the same extent, so it is probable that this smaller attack was not due to the host's belonging to a different species.

Cause of the Retardation of the Histoblasts.

In order to arrive at some definite conclusion as to why the histoblasts of a parasitized larva should not develop normally, it will be of advantage to review briefly some of the theories which have been advanced to explain the normal development of these discs, and at the same time to consider the facts which have been brought to light by the study of several cases now on record in which the discs have developed with abnormal rapidity, thus producing larvæ which possess characters that normally do not make their appearance until the pupal or adult condition is reached. This abnormal condition has been termed "prothetely" by Kolbe (1903).[1] For the abnormally retarded condition as produced in the *Simulium* larvæ by the presence of *Mermis* I should like to suggest the word "metathetely."[2]

The earliest report of prothetely was made by Heymons ('96). In this case larvæ of *Tenebrio molitor* L. were found which, when mature, proved to be abnormally developed as follows:

1. The meso- and meta-thorax possessed expanded lateral portions of the tergites, which resembled the wing-pads of the pupa, though they were not folded under the body as in the latter, but were directed backwards.
2. The antennæ had additional incompletely segmented joints, thus approaching the 11-jointed adult condition.
3. The abdominal tergites were modified so that they resembled the tergites of the pupal abdomen.

These larvæ were raised in the mealworm cultures of the

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[1] Προθεῖν, to run before, and τέλος, the completion.

[2] Μεταθεῖν, to run behind, and τέλος, the completion.

Berlin Zoölogical Institute, but no statement is made as to whether conditions during development were quite normal. Heymons concludes his paper by suggesting that the abnormal development described above is due entirely to an accelerated development of the histoblasts, but makes no suggestions as to the cause of the acceleration.

In 1903 Kolbe reported and figured an interesting case of prothetely in the larva of *Dendrolimus pini* L. He received the larva when in its fourth moult, at which stage it had the following characters:

1. The larval antennæ were replaced by elongate antennæ showing simple primary division into about seven segments.
2. The larval thoracic legs were replaced by three pairs of jointed legs possessing all the adult parts, namely, coxæ, trochanters, femora, tibiæ and tarsi.
3. The mouth parts were modified.

Kolbe points out that all these organs were in an immature adult, or true *pupal* form, thus showing, it would seem, that the development was quite normal, but simply accelerated. Winneguth succeeded in breeding from a similar larva an adult which hatched as a small male that was apparently quite normal except for its dwarf size. These abnormal larvæ were produced from an artificially hatched generation kept indoors and from parents which did not hibernate.

Hagen ('72) gives an account of silkworm larvæ obtaining wings before pupation, which condition was accompanied by other abnormalities as follows: The head was small and had two small facetted eyes, and the thorax became modified but the abdomen remained in the normal condition of a larva in the fourth moult. The fore wings were long and narrow and rather more gray than usual, while the hind wings were short and narrow. As this anomaly occurred frequently and was therefore liable to be of economic importance, its causes were investigated by Majoli who came to the conclusion that it was due to the larvæ being kept at a temperature above the normal.

A case similar to that of Heymons was described by Riley ('08) from another coleopterous larva, *Dendroides canadensis*, which was bred by a student at Cornell University.

It will be seen from these cases that in every instance they occurred in artificially reared larvæ, and it is probable that the prothetely was due to an increased temperature, perhaps with the aid of abundant food, in some way hurrying on the development of the histoblasts.

This fact suggests that the histoblasts may be caused to develop, though at a much slower rate than the larval organs until pupation, by some enzyme secreted by the insect and that they can develop only as fast as this stimulant is formed. Its supply thus acts as a regulator. An increased temperature is in most cases advantageous to enzyme action and in this case it is probable that it either causes more of the enzyme to be secreted or stimulates the action of the amount already available.

Dewitz ('05), after numerous experiments on retardation and acceleration of development and pupation arrived at the following conclusions: Development and pupation are caused by enzymes which are not very evident in the early larval stages. They, however, increase with the growth of the larva until its pupation, at which period they are at their maximum strength; they then begin to diminish, till at the end of the pupal period their action entirely ceases. He also states that the enzyme action can be hindered by the presence of another body; and that by obtaining an enzyme of an increased strength before pupation development can be abnormally accelerated.

It is evident that the cells of the histoblasts are caused to develop by a different stimulus from that which causes the development of the larval organs, for in the former case development is very slow during the larval stage, when the latter organs are developing very rapidly, and it is only when these have reached their limit of development at pupation, that the adult organs begin to develop with any rapidity.

This suggests that there may be in the insect body two sets of enzymes which one may term "larval" enzymes and "imaginal" enzymes. These are sufficiently different so that conditions which cause the acceleration or retardation of one of them need not necessarily have any effect on the other. If this be so, one has a probable explanation of prothetely and also, as I shall attempt to show in the following paragraphs, of metathetely.

At first sight one would suppose that the retardation in development of the histoblasts in parasitized *Simulium* larvæ is simply due to a lack of proper nourishment for these organs, since the larva, besides having to supply the requirements of its own developing larval tissues, has also to supply the demands of its fast-growing parasite. This may be true to a certain extent, but later observations, when another parasite is also present, indicate that there must be some other more potent factor whch accounts for this inhibition. This second parasite is a Sporozoön which, owing to the vast numbers in which it occurs in a single host, is far more bulky than the worm and must, one would imagine, make far greater demands on the resources of its host. In this case, however, the histoblasts are usually unaffected in size, though in many cases they are distinctly smaller than normal. Two individuals, however, were seen in which the histoblasts were minute. On dissection it was found that, in each case, a small worm measuring only some 7 mm. was living embedded in the mass of spores, and it was evident that this minute worm was responsible for the retarded condition of the discs, even though it had evidently absorbed very little nourishment. One must, therefore, in all probability look to some *toxin* secreted by the worm as the cause of the inhibition of development in the discs.

The researches of Verson and Bolle, as quoted by Fischer ('06), proved in the case of lepidopterous larvæ that in their early stages their body fluid is alkaline and that this alkalinity decreases as the larva matures. This would suggest that an alkaline condition encourages the growth of larval tissues whereas acidity, or the absence of alkalinity, permits of the development of the adult organs. Hence the histoblasts would develop but slowly till the larvæ are nearing maturity when the decreased alkalinity of the blood allows the "adult" enzymes to stimulate the cells of these discs to rapid division. I have been unable to find any account of the excretions of *Mermis* or of closely related Nemathelminthes, but should they be proved to have an alkaline reaction the probability of the above contention would be very greatly strengthened, for here we should have a case of the alkalinity of the system being maintained in maturing larvæ, and thus preventing the normal, though slow; development of the adult organs, without affecting the larval organs to any great extent, except in so far as they are kept well supplied with alkaline fluids and are thus capable of developing to their utmost extent. This would account for the somewhat larger size of parasitized individuals.

It may be, however, that the *Mermis* does not actually secrete an alkaline substance, but brings about an increased alkalinity in the body of its host by absorbing whatever acids are formed in it. The probability of this being the true condition is increased by the fact that closely related worms live in acid media, such as vinegar or sour paste (*Anguillula aceti* Ehrbg.), which would point to the fact that the worm requires, and absorbs, acids during its development. In either case the effect on the host would be the same in that there is a reduction in the activity of the acids which appears to be essential to the development of the histoblasts. Whether this is the true explanation or not, it is certain that the presence of the worm does have a direct inhibitory effect upon the development of the imaginal organs, but does not have a similar effect on the larval tissues.

A suppression of pupal and adult organs will naturally be of advantage to the parasite for two reasons:

1. Nourishment is not required for building up these tissues and therefore more will be available in the body cavity of the host.

2. The maturity of the host will be deferred thus giving the parasite a longer life, should it require extra time for development. In most of the observed cases, however, the worm killed its host, by emerging before or at about the same time that the uninfested larvæ were pupating.

A similar though less marked case of metathetely due to parasitism by *Mermis* has been described and figured by Mrázek ('08) in the queens of a European ant (*Lasius alienus*). In this case the parasitized larvæ matured and produced adult ants which were normal in all external characters with the exception of a great reduction in the size of the wings. Through the kindness of Professor Wheeler I have been able to examine some similarly parasitized specimens of a closely related American species (*Lasius neoniger*) in order to see whether the development of the legs had been in any way affected but a careful comparison of the measurements of the legs of the parasitized ants with those of healthy specimens failed to reveal any inhibition of their development on account of the *Mermis*, although the wings, which in normal ants measured some 10–11 mm. in length were reduced in the parasitized individuals to 6–6.5 mm.

In the case of *Simulium* larvæ, as before stated, the development of the legs also is inhibited by the presence of *Mermis*, though comparative measurements of the wings and leg histoblasts in healthy and parasitized larvæ show that whereas in the former case the wing histoblast covers about four times the area covered by that of the mesothoracic leg, in parasitized larvæ these histoblasts bear a relation to each other in size of about 2.5 : 1, showing that the wing histoblast suffered a greater inhibition in development than did that of the leg.

A further interesting case of *Mermis* parasitizing ant larvæ was described by Wheeler ('10), in which case the worker larvæ of *Pheidole commutata* were parasitized, and resulted in the adults of such larvæ not only possessing all the normal "worker" characters perfectly developed, but owing to excess of feeding on account of their constant hunger these "worker" larvæ developed, when mature, characters such as the ocelli which are normally only found in the sexual ants.

A Sporozoön Parasite of Simulium Larvæ.

While dissecting out worms from a batch of larva taken on March 30, I chanced to cut open one larva which from the whiteness of the abdomen I took to contain a worm, but was much surprised to find that the body cavity was closely packed with a white substance which had the appearance of cotton wool. A little of this substance, however, when smeared on a slide was seen to be composed of countless organisms as illustrated in Plate V., Fig. 14. My first impression, very naturally, was that these were spermatozoa, which they resemble very closely in general outline. It was, however, very difficult to imagine to what possible organism these spermatozoa could belong. The larva itself could surely not produce them; but if not the larva what then? The only explanation seemed to be that they were formed by one of the worms, which could not then be *Mermis* but must be a new form closely related to *Allantonema* or *Sphærularia*, in the females of which the uterus becomes protruded and is finally many times the size of the original worm.

I naturally visited the stream in order to obtain more specimens suffering from this disease, only to find that a quantity of oil which had been flowing down the stream for some days past had succeeded in killing off all the larvæ in that neighborhood. It was therefore necessary to find a place further up the stream, above the contamination, where the larvæ were living. About half a mile's walk led to such a place, situated under a stone arch, in which the larvæ were present literally in thousands. They formed great masses covering the whole surface of the rocks. An examination of a few rocks soon showed that the *Mermis* was very plentiful here, but it was some time before I found specimens with the curious white abdomens for which I was searching. I chanced, however, to pull up a piece of water weed that was floating in a swiftly running swirl and here I found quite a number of individuals, some showing immense abdominal swellings due to the parasite. On examining a few small specimens I found that the parasite was more numerous among them than among the larger individuals; a closer examination of the rocks showed that these small parasitized individuals were quite commonly scattered among the larger healthy ones. A quantity of material was taken back to the laboratory to be studied on the same lines as that adopted for the *Mermis* parasite.

External Effects on the Larva.

The first effect noticed in badly infested larvæ is the immensely distended abdomen. In some cases as in that illustrated in Plate V., Fig. 12, the apex of this region of the body was three to four times its normal size. Unlike the *Mermis*, this parasite is not confined to the ventral portion of the body but entirely surrounds the caudal extremity of the mesenteron, and small detached colonies were not infrequent in the thoracic region. The Malpighian tubules, however, can usually be seen on the surface of the mass and are plainly visible through the tightly stretched skin, which is quite transparent in places and appears to be on the point of bursting.

The length of the larvæ next claims attention. No larva containing this parasite was found to be exceptionally large; whereas, as before mentioned, many were extremely small, measuring some 2.75 or 3.5 mm. at a time when all normal larvæ measured some 9 mm. or more. This may possibly require a similar, though opposite, explanation to that suggested on page 281 to account for the large size of the individuals affected by the *Mermis*.

In confinement these small specimens were extremely restless, they were continually twisting about and moving over the sides and bottom of the jar with a peculiar jerky movement. The larger, and more heavily parasitized, larvæ on the other hand were more sluggish though not more so than the healthy larvæ.

The effect of these parasites on the histoblasts is very hard to state with any certainty. Fig. 13 is an illustration, made with a camera lucida, of the histoblasts of a nearly mature individual. In this case the parasite was confined to the abdomen. It will be seen that the discs are but half developed (compare with Fig. 6). In other cases, however, the development of the discs was not, so far as could be seen, affected in any way till pigmentation of the respiratory filaments commenced. Then it was noticed that the entire histoblast only turned a slate gray color instead of blackening at the apex of the filaments and finally over the complete disc. In other cases, however, the discs entirely blackened in a perfectly normal manner.

When colonies of parasites are located in the thorax, as is not infrequently the case, the histoblasts are materially decreased in size, while in the very small specimens development appeared to have been arrested. This is hardly surprising. My general conclusions were that the parasite affected the histoblasts but little, though the larvæ rarely advanced so far toward maturity that the respiratory filaments became pigmented. It should be noted that the power of spinning silk was in no way affected by the presence of this parasite.

Nature of the Parasite.

While visiting other localities I made numerous observations on the larvæ to be found in the streams, and was much surprised to find that species of the parasite were extremely common and that in almost every brook visited some of these conspicuously distorted individuals were to be found. On making microscopical examinations I observed that the organisms taken from different localities varied very much in form and apparently represented different species. A list of these different forms and their hosts is appended:

1. Ovoid bodies bearing a "flagellum"-like organ varying in length from about that of the "body" to three times its length (Plate V., Fig. 14). Host: *Simulium hirtipes*. Habitat: Forest Hills and Blue Hills, Mass.

2. Bi-annulated ovoid bodies bearing a "flagellum," which is never much longer than the "body" (Plate V., Fig. 15). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation, Mass.

3. Ovoid bodies having the "flagellum" replaced by a transparent flattened disc (Plate V., Fig. 16). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation, Mass.

4. Simple ovoid bodies destitute of all appendages (Plate V., Fig. 17). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation and Blue Hills, Mass.

The following characters were common to all of these organisms:

1. They were all similar in size.

2. Under the highest power of the microscope they had a faint olive greenish tinge.

When in a fresh condition absolutely no internal details were visible and in specimens which had been killed, fixed and stained very little more could be seen. In many of the first or "spermatozoid" type a darkened central area indicated the presence of a very large nucleus, while a smaller dark spot just before the base of the flagellum might be taken for the nucleolus.

Many of the "simple ovoid" type, although but slightly staining, showed a great shrinkage of the internal substance; otherwise nothing could be seen in them. The specimens with a flattened disc, and those bearing two annuli and a flagellum, also showed practically no differentiation. Methyl green and various hæmatoxylin combinations were tried without success.

Besides these various forms, no two of which were observed to occur in the same individual, another form of cell (Plate V., Fig. 18) was found in much smaller numbers, but possibly in some way connected with them, for it was seen in association with each form, but was not found in healthy larvæ. This cell, which varied much in diameter from but little more than the numerous "spores" to three or four times the length of their longest axis, was apparently globular in form, and in a fresh state was very transparent and could only be discerned with difficulty. The following characters, however, were seen. The substance of the cell was finely granular, and often contained a number of large transparent globules. When fixed and stained the only differentiation was that of a large dark mass, apparently the nucleus (Plate V., Figs. 18, *a* and *b*). In some cases these cells seemed to be dividing (Plate V., Fig. 18, *c*).

In the face of all these diverse types of cell, which live in precisely the same way, it is evident that the forms first found are not spermatozoa, and further the fact that the flagellum is replaced in one "species" by a flattened disc eliminates the possibility of their being *Flagellates*. It is therefore probable that one must look to the *Microsporidia* among the *Sporozoa* as the group to which these bodies belong. It is further noticed that many of the forms are very similar to those met with in the genus *Glugea* to which the well known *pébrine* disease (*G. bombycis*) of silk-worms belongs. One must therefore conclude that it is a pebrine-like disease, which is killing off a high percentage of the Simuliid larvæ in the neighborhood of Boston, though on account of the vast difference in structure and mode of life of the two hosts the life history of the parasite in the *Simulium* larvæ is very unlike that described by Pasteur ('70) and Stempell ('09) in their work on the disease in silk-worms.

LIFE HISTORY OF THE PARASITE IN ITS HOST.

As in the case of *Mermis*, I know very little about this, since I did not discover it till the final stages of the larva were being approached, and in every instance but one it was apparently in exactly the same condition, namely, the "spores" were all formed and were simply awaiting the death of their host and its resulting decomposition to escape into the water.

The one exception, however, was that of a larva found on April 17. It was one of the first discovered, in the abdomen of which could be seen, with a dissecting lens, large white bodies floating in the blood plasma. On dissection these bodies proved to be rounded masses of flagellate spores, a few of which had become disengaged and were apparently moving about by their own impetus in the blood plasma. As, however, I have never on any subsequent occasion observed such a movement among these spores I am inclined to believe that this was simply a Brownian movement, which I have distinctly recognized in later examinations. Though I searched carefully for other larvæ with parasites in a similar stage I was unsuccessful.

In serial sections of parasitized larvæ similar effects to those exhibited when *Mermis* is present, are noticed in that the fat-body is much reduced (Plate IV., Fig. 10) and the sexual organs could not be detected, whereas the spinning glands and musculature apparently remained unaffected. The parasite probably enters the host through the alimentary tract for in all cases of infected larvæ it was found that the mesenteron was distorted in one or more places showing distinct hypertrophy as if the cells had been badly irritated but had healed over again often permitting the spores to pass through into the body cavity (Plate IV., Fig. 10, *e*). In one case a small colony of "spores" was found inside the alimentary tract, but it is quite possible that these had been recently taken in with the water, as parasitized larvæ were constantly dying in the colony, and any material, floating in the water such as liberated "spores," would be caught by the cephalic fans of still living larvæ.

In all recorded cases of Microsporidia parasites it has been noticed that the "spores" live, at least during their early stages, inside some body tissue of the host. An example of this is to be found in pébrine of silk-worm larvæ, in which case the spinning glands are the main seat of attack. It must be borne in mind, however, that an attack upon these organs in the larva now under consideration would, in all probability, result in the early death of the host, for it is only by means of the much strengthened silk threads that the larva is enabled to maintain in rapid streams the perpendicular position which is essential to obtaining food, while at the same time the larva has to depend to a large extent on these threads for retaining any foothold on the rocks, for when moving its position, the adhesive disc of the proleg frequently loses its grip and the larva is washed clear of the rock. Very rarely, however, the anchoring threads, which are always present, break so that the larva is able slowly to draw itself once more to its support. Sections of the *Simulium* larva disclose the fact that there are very few other tissues in the body. The muscular system is much reduced, and the only tissues available for attack without rapidly killing the host are the fat-body and the sexual organs, and I am under the impression that it is the latter which are usually the original seat of attack. In frontal sections of a very young larva taken during March, only one testis could be found, but on the other side of the body a small mass of minute cells, taken at the time for small œnocytes, was situated a little back of the normal position of the missing testis. The cells are too small to show any structures but it is possible that this is a diseased testis to which the spores made their way as soon as they entered the body cavity. Later sections also show traces of a very thin membrane investing the mass of "spores."

I kept a large number of diseased larvæ in running water, hoping to see some of them pupate, but in every case they died, and soon liberated the spores, whereas many of the healthy larvæ, which were approaching maturity, pupated in captivity. It is thus evident that heavily parasitized larvæ never pupate, but die in the stream, liberating their countless spores in the water. What happens to these spores I have been unable to ascertain. Larvæ were allowed to die in distilled water and the liberated organisms were examined at intervals, but though they strongly resisted decomposition they never showed signs of movement or altered condition. Sections of pupæ and adults obtained from badly infested localities failed to reveal any cases of the disease being carried beyond the larval stage. It is therefore probable that the disease is not hereditary as is the case with pébrine, and as before stated it was seen that the presence of this parasite, in every case examined, apparently resulted in castration of the host. It is, however, possible that among the vast numbers of larvæ a few were only slightly parasitized and did not have their sexual organs entirely destroyed. Such individuals should be capable of pupation and might in that way carry the disease over as in the case of pébrine. An examination of several hundreds of larvæ did not reveal one in which such a condition was possible.

Were there overlapping generations of larvæ the maintenance of the parasite could be more easily understood, but the spring brood pupated and hatched during the first half of May, and there are not at the time of writing any signs of more eggs being deposited on the rocks. It thus seems that there must be a secondary host in which this parasite passes the summer.[1]

THE PERCENTAGE OF INFESTED LARVÆ.

The number of infested larvæ varied to a great extent in different streams. In the part of the stream in Forest Hills where the parasites were first noticed, less than 1 per cent. of the larvæ were parasitized, half a mile further up the stream a little under 40 per cent. were infested, while in the Stony Brook Reservation where two types of "spores" were present among the parasitized larvæ, between 70 and 80 per cent. of the individuals were to be seen with the immensely distended and whitened abdomens which proclaimed them to be suffering from this fatal disease.

It will thus be seen that should this disease together with the previously described *Mermis* parasite, prove to exist as abundantly in other localities as it has during the past spring in the vicinity of Boston it must be of considerable importance in the natural control of the black flies which are such an annoyance to man and beast, especially in more tropical regions, and if the supposed secondary host of the Sporozoön does not prove to be a fish or animal of any value it should be possible to infect streams where Simuliid larvæ breed, and diminish their numbers very largely without the danger of poisoning the fish by applying oil or other substances to the water.

SUMMARY.

Simulium larvæ, which are found in vast numbers in small rapid streams around Boston, are seen to feed by standing perpendicularly on rocks to which they are attached by a strong anal adhesive disc, and kept in position by silken threads secreted by the salivary glands. While thus anchored they spread out a pair of cephalic fans which act as strainers and collect small particles of food from the water. The head capsule is moulted independently of the body cuticle and exposes a new capsule which is at first white with a few dark spots on the vertex, but which rapidly becomes uniformly darkened all over. The thorax bears unusually well defined and large histoblasts of the imaginal wings, halteres and legs, and also on either side a histoblast of the pupal respiratory filaments, which by turning black when the larva is mature becomes very conspicuous at this stage of growth. The larvæ are infested by two parasites, namely a *Mermis* and a Sporozoön, both of which live in the body cavity.

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[1] I have since July 31 noticed isolated specimens of a different species of larva in one of the streams where the parasite abounded in the spring brood, but have not found any in which there were signs of the parasite being present.

The *Mermis* does not affect the larval development to any extent, except by slightly increasing its size, but it inhibits the development of the histoblasts to such an extent that pupation becomes impossible.

The embryo worms are probably caught by the cephalic fans of the larvæ and pass into the alimentary tract, through the walls of which they bore and live in the body cavity of the host till the latter matures. They then rupture the abdominal cuticle and pass into the water where they live a free life under stones in the bed of the stream. The number of worms contained by a single larva is usually only one, but as many as twelve have been found. A single worm measures 3 cm., which is about three times the length of the host. In some streams 25 per cent. of the larvæ were infested with this parasite. Parasitized larvæ never pupate, but are killed by the worms when they escape.

The retardation in the development of the histoblasts is the opposite condition to that met with in prothetely which is usually caused by keeping larvæ at an abnormally high temeprature. This probably results in an increased supply of the enzymes which cause these histoblasts to develop. The *Mermis* apparently excretes some substance which lessens the supply or action of these enzymes and leads to metathetely.

The Sporozoön parasite occurs in several forms in different localities. All these forms, however, live in the same way and appear to be related to the pébrine disease of Lepidoptera. The body, especially near the apex of the abdomen, becomes much distorted and swollen on account of its interior being closely packed with a white wooly material which on dissection is seen to consist of countless "spores" of minute size. Such parasitized larvæ are usually rather smaller than healthy individuals, but the histoblasts do not appear to be much affected. The parasite apparently enters the body cavity in the same manner as that described in the case of *Mermis*. Evidence of this is seen in a hypertrophied condition of parts of the mesenteric wall. From here it seems to pass to one or both of the sexual organs which are destroyed and become the nuclei for the great mass of spores which eventually fills the abdomen. The parasitized larvæ in this case also were never observed to pupate but died when mature. The spores are liberated by a rupture of the abdominal wall soon after the death of the host and pass into the water, after which stage they have not been seen. Up to 80 per cent. of the larvæ in some streams were found to contain large masses of this parasite but no cases of slightly parasitized larvæ were observed. There has been no second brood of *Simulium* larvæ this year, so it would seem that if the parasite is to appear next year there must be a secondary host in which the summer is passed.

POSTSCRIPT.

The foregoing account of the Sporozoid parasite of *Simulium hirtipes* was very kindly reviewed for me by Professor G. N. Calkins, of Columbia University. From mounted specimens of the spores sent him he confirmed my opinion that these represent some species of Myxosporidea, and drew my attention to a paper by Louis Léger ('97), which I had overlooked because of its not being catalogued under *Simulium* in the cards of the "Concilium Bibliographicum" in the Cambridge library. In this paper a new species of *Glugea* parasitizing the larvae of the European *Simulium ornatum* is described as *G. varians*. The notes on this species are in brief as follows: The abdominal region of the infested larva is greatly distended and contains large masses of a free parasite in the form of opaque, milky white, irregular sacs.

Some larvæ contain but one mass whereas others contain two to four. The muscles are unaltered, but the fat body is much reduced. The alimentary canal always appears to be contorted. In only one case had the Microsporidea failed to sporulate and they then formed a swelling on the external intestinal surface. The sacs contain countless ovoid, refractive spores, which, when treated with iodine, show a filament 15–20 times as long as their longest diameter. Spores of two sizes are present, the smaller measuring 4–5μ, the larger 8μ. In a subsequent note written in collaboration with Hagenmueller ('08) Léger states that the spores are sometimes present in a polysporic and sometimes in an octosporic arrangement. These authors also refer to a similar parasite in the larvæ of *Tipula gigantea*.

From the foregoing notes it will be seen that the disease which occurs in *S. hirtipes* is very similar in its main features to that described by Léger, and I do not hesitate to regard the organism responsible for its occurrence as a closely related form. Professor Calkins is inclined to consider the various forms I have described as belonging to a single species. For this I would propose the name *Glugea polymorpha* sp. nov. Future investigation, however, will quite possibly show that the various forms occurring in different localities are not all representatives of the same species, since numerous dissections of diseased larvæ showed that certain types of spores were peculiar to different localities even though present in two different species of *Simulium* larvæ.

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EXPLANATION OF PLATE I.

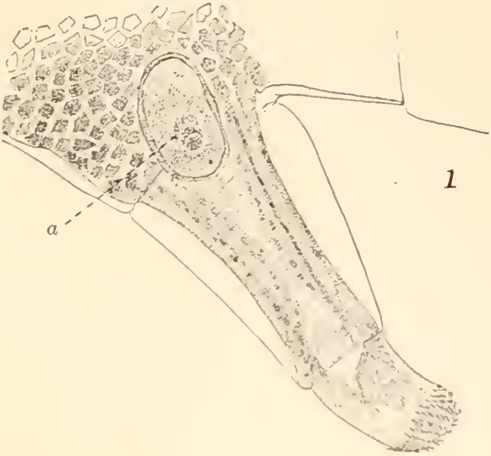
FIG. 1. Prothoracic leg of a *Simuliid* larva showing the histoblast (*a*) at an early stage of development.

FIG. 2, *a*, *b* and *c*. Types of spotting on the head of a recently moulted larva of *Simulium hirtipes*.

FIG. 3. A single rake from the cephalic fans.

FIG. 4. Profile of thorax of a half-matured larva showing the histoblasts. *a*, respiratory organ histoblast (pupal organ); *b*, wing histoblast (adult organ); *c*, halterer histoblast (adult organ); *d*, *e* and *f*, pro-, meso- and metathoracic leg histoblasts (adult organs).

FIG. 5. Ventral view of a *Simulium* larva with *Mermis* parasites in situ.



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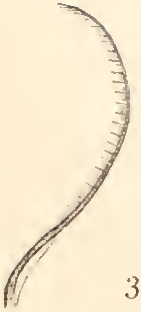

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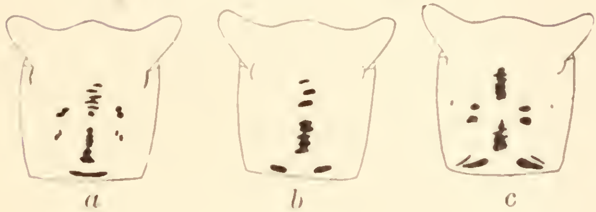

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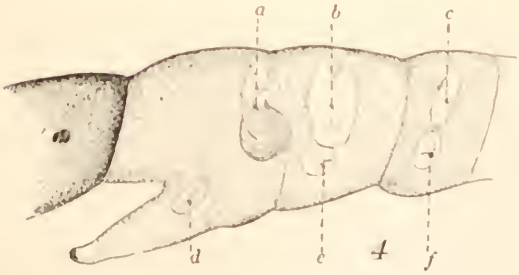

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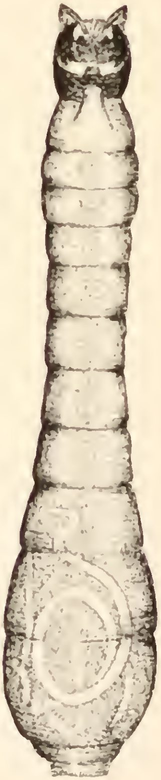

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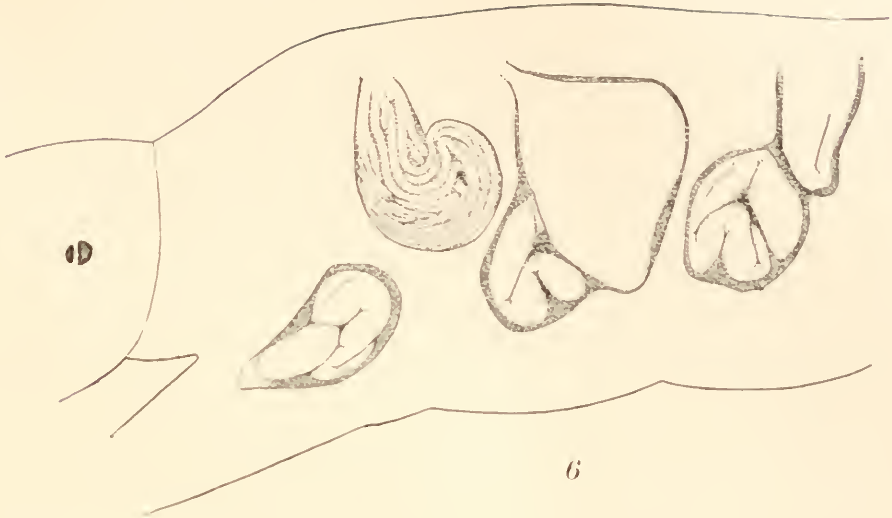
Explanation of Plate II.

Fig. 6. Histoblasts of a healthy full-grown larva measuring 10.5 mm.

Fig. 7. Histoblasts of a larva parasitized by *Mermis* measuring 10.5 mm.



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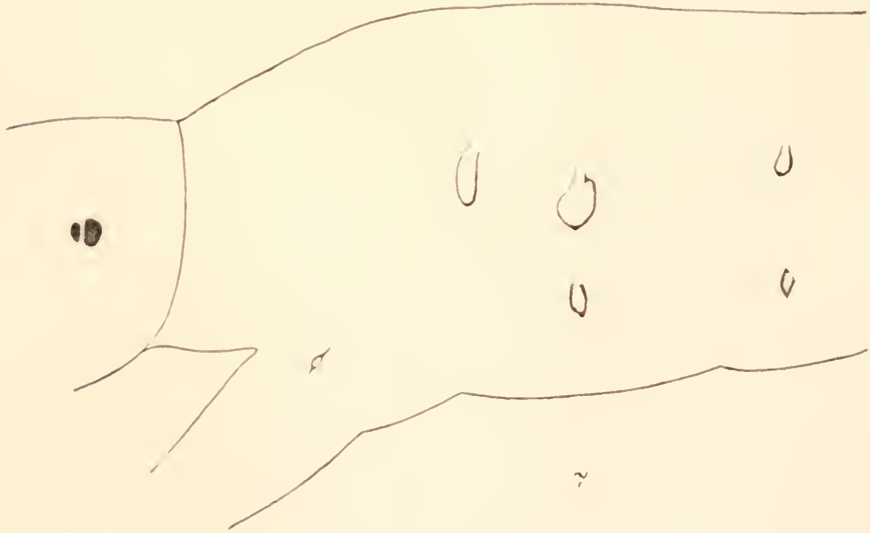

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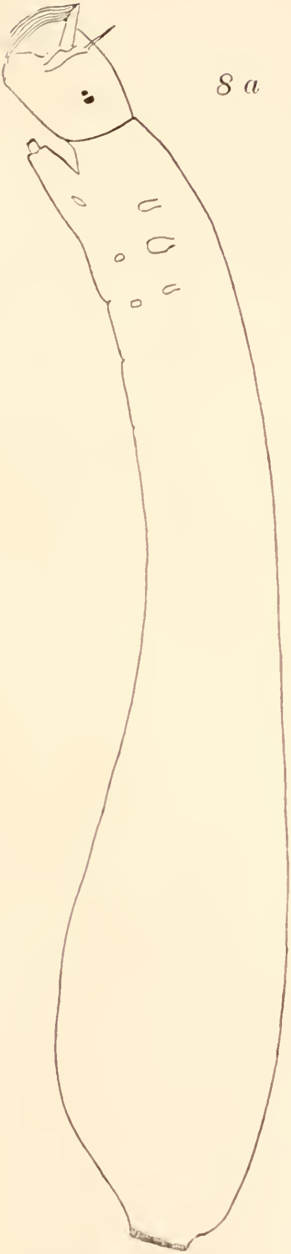
Explanation of Plate III.

Fig. 8*a*. Average size full-grown parasitized larva of *Simulium hirtipes* measuring 11 mm.

Fig. 8*b*. Average size mature larva of *Simulium hirtipes*, measuring 8.5 mm. showing darkened pupal histoblast, *a*.

Fig. 11. Median sagittal section of a half-grown healthy larva to show the alimentary tract. *b*, the normal position of the spinning glands, which extend backwards from the pharynx on either side latero-ventrally to the alimentary canal, doubling back on themselves at about the point *b*, where one has been cut in cross section; *c*, the normal quantity of fat body which increases still more as maturity approaches; *d*, one of the pharyngeal glands. (This is not seen in an exact median section, as the two glands are narrowly separated medially.)



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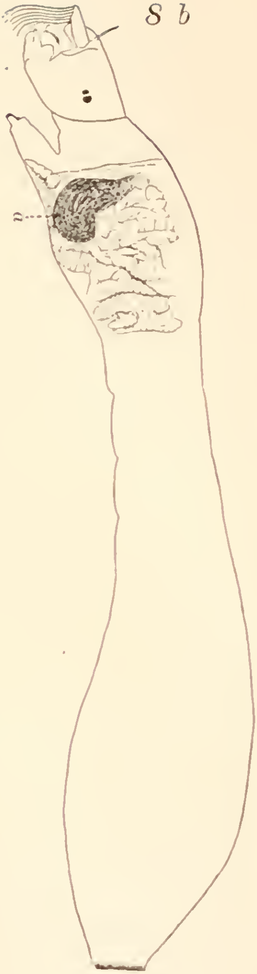

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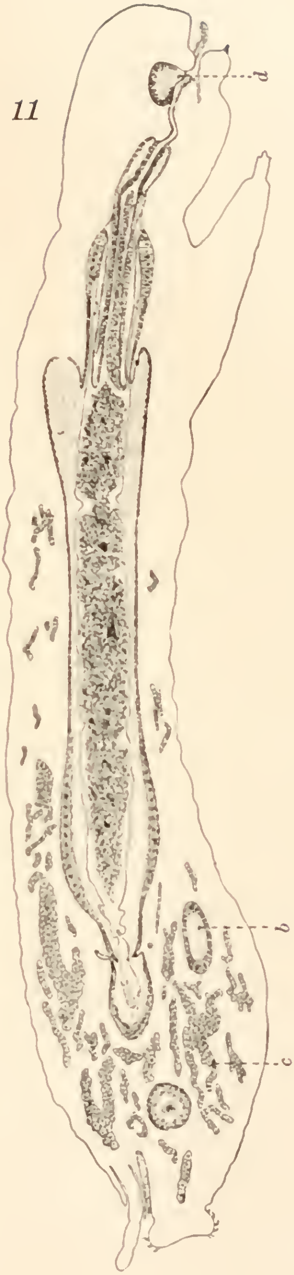

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EXPLANATION OF PLATE IV.

FIG. 9. Median sagittal section of a larva parasitized with *Mermis* sp. to show the somewhat displaced alimentary tract, and *a*, the coiled up worm; *b*, the displaced spinning gland, a short longitudinal section of which has been cut; *c*, the much reduced fat-body; *d*, one of the pharyngeal glands.

FIG. 10. Median sagittal section of a larva parasitized with a sporozoön to show *a*, the mass of spores, a few of which are shown enlarged; *b*, the spinning gland; *c*, the much reduced fat body; *d*, one of the pharyngeal glands, and *e*, the somewhat distorted wall of the alimentary tract.

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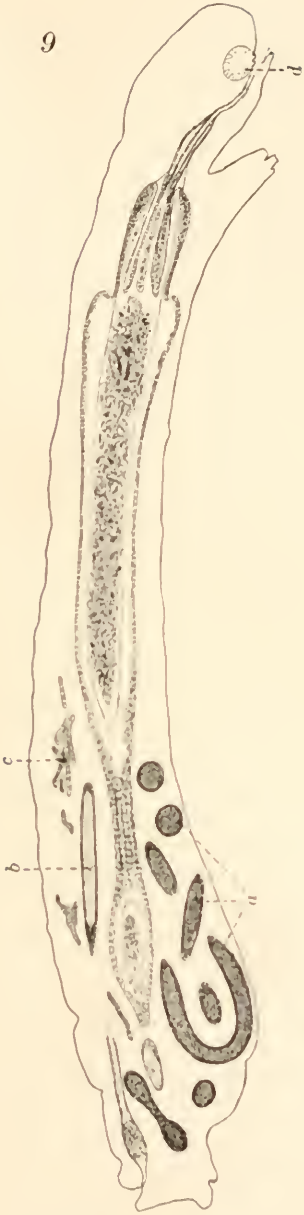

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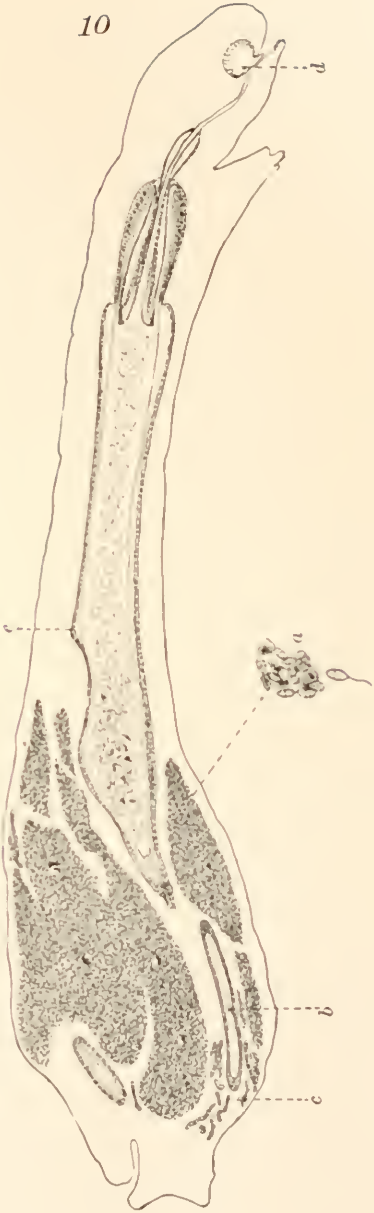

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EXPLANATION OF PLATE V.

FIG. 12. Dorsal view of a *Simulium* larva with *Sporozoid* parasites in situ. Compare with Plate I., Fig. 5, which is normal in shape.

FIG. 13. Histoblasts of a larva parasitized with the *Sporozoa* measuring 9 mm. Compare with Plate II., Fig. 6.

FIG. 14, *A*, *B* and *C*. Simple "flagellate" type of parasite, ×4,000.

FIG. 15, *A* and *B*. Annulate "flagellate" type of parasite, ×4,000.

FIG. 16. Type of parasite with "flagellum" replaced by a flattened disc, *A* showing surface view of disc, ×4,000; *B* showing side view of disc, ×4,000.

FIG. 17, *A*, *B*, *C* and *D*. Simple ovoid type of parasite, ×4,000.

FIG. 18, *A*, *B* and *C*. Other bodies found in small numbers among the "spores," ×4,000. Many are larger in proportion than those illustrated. *C* shows one apparently dividing.

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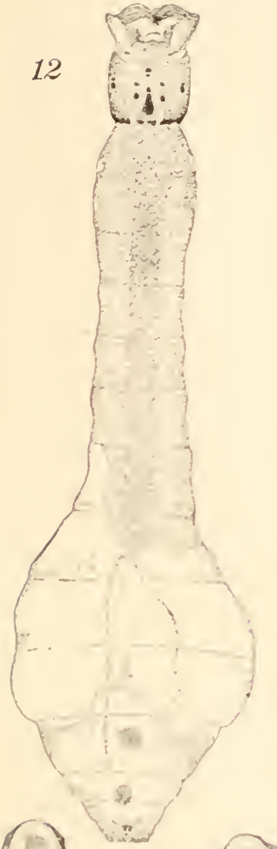

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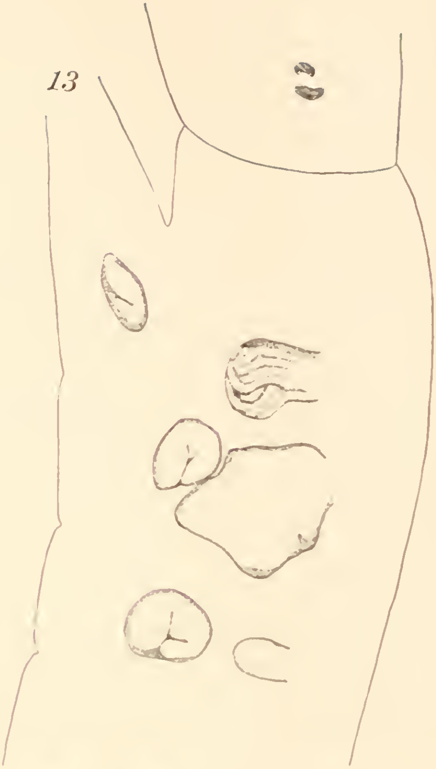

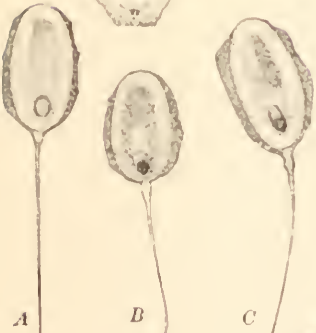

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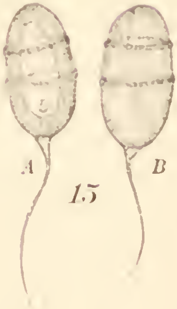

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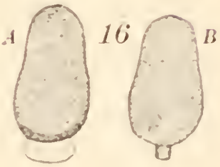

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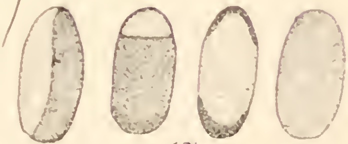

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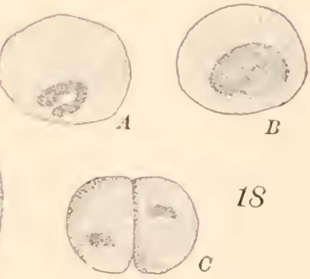

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Vol. XXI. *November, 1911.* *No. 6.*

BIOLOGICAL BULLETIN

THE PERSONAL EQUATION IN BREEDING EXPERIMENTS INVOLVING CERTAIN CHARACTERS OF MAIZE.[1]

RAYMOND PEARL.

In the summer of 1908 some experiments in the cross-breeding of certain types of maize were begun by the writer and his former colleague, Dr. Frank M. Surface. The present paper has to do with a part of the results obtained by crossing a white sweet variety (♂ parent) with a yellow dent variety (♀ parent). Both varieties used were "pure," in the sense that each bred true to the general type to which it belonged. The history of the sweet variety used has been detailed in another place[2] and need not be repeated here. The important thing to be noted at this time is that in its whole history this sweet corn used in the cross breeding experiments had never been known to produce any but *sweet* (sugary) kernels of an exceptional degree of *whiteness.*[3]

The dent corn used in the experiments was also of known history. A discussion of its history, and of the characteristics of the corn has been given elsewhere.[4] The essential point to be noted here is that during a long period of years it has never produced anything except *starchy* kernels of a deep orange *yellow* color when ripe.

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[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 29.

[2] Pearl, R., and Surface, F. M., "Experiments in Breeding Sweet Corn," Me. Agr. Expt. Stat. Ann. Report for 1910, pp. 249-307.

[3] A pure chalky white or, put in the other way, the entire absence of yellow color, is an absolute essential of a high grade of corn from the packer's standpoint. The sweet corn here under discussion is regarded by expert packers as an exceptionally fine strain for their purpose.

[4] Pearl, R., "The Mendelian Inheritance of Certain Invisible Chemical Characters in Maize," *Zeitschr. f. Abst.- u. Vererb.-lehre*, Bd. VI., 1911.

The general results which follow the crossing of a yellow dent (♀) with a white sweet (♂) maize are well known. Yellowness of endosperm is dominant over "whiteness" of endosperm, and "starchiness" over "sweetness." Consequently the F_1 kernels are externally indistinguishable (in fact as well as in theory) from those of the pure yellow dent parent. These F_1 kernels planted give rise to plants bearing ears of which each should have four distinct kinds of F_2 kernels which ought, by theory, to occur in the simple dihybrid ratio, 9 yellow dent, 3 white dent, 3 yellow sweet, 1 white sweet.

The present experiments[1] entirely confirm in all essential respects this general Mendelian result. Certain novel points arose, however, in the course of the work, which led to the present investigation. These points may now be considered.

A large quantity of ears bearing F_2 kernels was raised. These ears were well matured. This was indicated both by their appearance and by the way the seed from them germinated. One of the assistants in the laboratory, Miss Maynie R. Curtis, undertook the sorting and counting of these F_2 kernels on an extensive scale. In this work the following situation immediately developed and was called to the writer's attention. While *in general* the F_2 kernels fell without any doubt or difficulty into the four classes or categories, yellow starchy, white starchy, yellow sweet and white sweet, yet there were a number of kernels on each ear that were extremely difficult of classification. These kernels were, in short, *intermediate* in respect to their external visible *somatic* characters, and might, in the individual case, be put with equal propriety into either of two classes. Into which class such an intermediate kernel would actually be put plainly depended upon the personal bias of the observer, rather than upon any peculiarity of the kernel itself. This result appeared to be of enough interest and potential significance to warrant a more extended and thorough investigation of the matter. The present paper deals with the results of such a study.

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[1] A detailed description of the conditions and manner of these experiments has been given elsewhere (Pearl, *loc. cit.*) and need not be repeated.

Statement of Problems and Plan of Investigation.

The problems with which this work is concerned may be summarily stated as follows:

1. To what extent is the personal equation of the observer a significant factor in the Mendelian ratios described for simple experiments with cross-bred maize? In other words, how closely would the different individuals of a group of competent biological observers agree in their classification and count of the *same* F_2 material from a maize cross involving such relatively simple and easily judged unit characters as color of endosperm, or chemico physical character of endosperm (starchy or sweet)?

2. Does somatic "intermediateness" in maize imply gametic "intermediateness"? In other words, do F_2 kernels which are intermediate *somatically* give rise to any different sort of progeny when planted than do kernels which belong clearly and indubitably to one or another of the well-defined *gametic* classes in F_2? If they are true "blends" in the Galtonian sense, they would certainly be expected so to do. If, however, they merely represent a phenomenon essentially like the incomplete or partial (somatic) dominance so frequently observed in Mendelian work, it would be expected that their progeny would differ in no essential particular from that obtained from somatically non-intermediate kernels having the same gametic constitution.

To test these questions the following plan was devised: Four ears bearing F_2 kernels were taken quite at random from a lot of about two bushels of such ears, which in turn was a random sample of a whole crop which included a much larger number of bushels. Each of these four ears was given an arbitrary number and was separately shelled, great care being taken to see that no kernels were lost. All the kernels from each ear were preserved together in a bag (or box). The shelling was done in the writer's laboratory in the presence of several workers, so that there can be no question whatsoever, that all of the kernels in each one of the four parcels originally grew upon the same ear.

Three of the ears so dealt with (Nos. 8, 9 and 10) were normal in every respect. Ear No. 11 was slightly abnormal in the respect that a fungus had attacked some of the grains, giving them a slight pinkish tinge in addition to their own proper color. This was especially noticeable in the case of the "white" sweet grains of the ear, because in a mature, dry sweet corn kernel "white" means merely the absence of any color (yellow or other). The grain is translucent and "not colored." Any extraneous color such as that arising from a fungus attack will be the more evident. The same considerations apply to the white starchy kernels, except that here the starch of the endosperm gives the grain a positive white color.

The kernels from each of the four ears having been separately shelled and preserved as described, fifteen persons (including the writer) were asked to sort the kernels of each ear into the four categories, yellow starchy, white starchy, yellow sweet and white sweet, and then count and record (on blanks provided for the purpose) the number of each sort found. Four small vials containing *typical* kernels of each sort were given to each observer as comparison samples. The only instructions given the observers were:

1. To sort and count the material *independently*.
2. To open and handle only one parcel of seed at a time.
3. Not to lose a kernel.
4. To count correctly, *i. e.*, to make sure that the total numbers of kernels counted tallied with the total numbers in the parcel, which numbers were set down on the blank for each ear.

Especial pains was taken to insure that no observer (with the exception of Nos. VI., VII., VIII. and XI.) should know, in advance of his count, the nature of the experiments which gave origin to the material, or the expected Mendelian ratio between the several classes of kernels. No observer[1] was, of course, allowed to see the results of the counts by others until after his own had been completed. In short every effort was made to insure in all possible ways that the counts tabled should be the unprejudiced, unbiased, independent and purely objective statements of the opinions of a group of competent biological observers as to the proper classification of the F_2 kernels from these four ears of maize.

We may next consider the observers who took part in this work. At the outstart the writer wishes to express his indebted-

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[1] With the single exception of No. XI., and in this case it was some *months* later that his own counts were made.

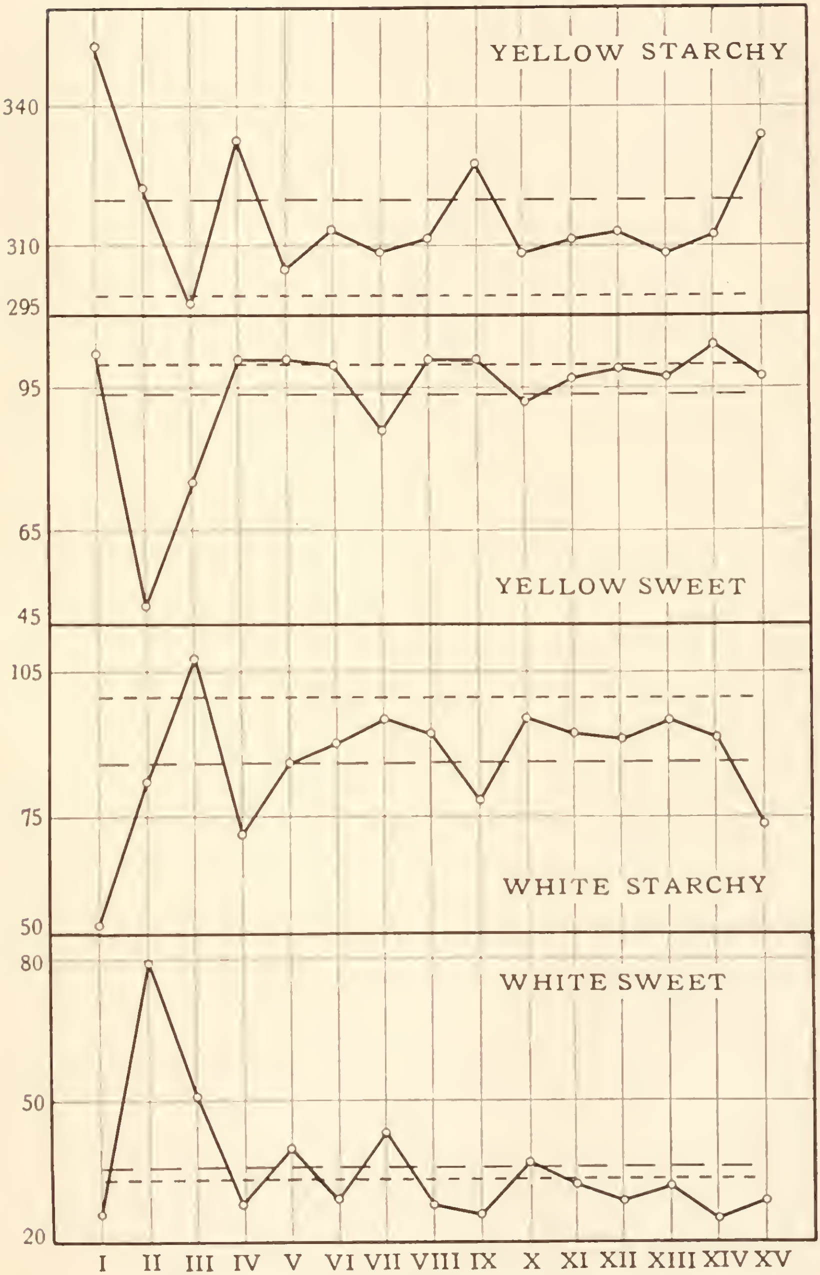

FIG. 1. Diagram showing the count of the different observers of each of the four classes of kernels for ear No. 8.

ness to all of those who coöperated in the investigation, and his appreciation of the painstaking interest and care given to the sorting and counting by all. Table I. gives the name, academic degree and official position of each of the coöperating individuals. For convenience of reference in the paper each observer has been assigned a Roman numeral.

Table I.

List of Observers Coöperating in the Present Study.

No.	Name.	Academic Degree.	Official Position.
I.	W. J. Morse.	M.S.	Plant pathologist, Maine Experiment Station.
II.	C. E. Lewis.	Ph.D.	Associate plant pathologist, Maine Experiment Station.
III.	G. E. Simmons.	M.S.	Professor of agronomy, University of Maine.
IV.	M. E. Sherwin.	M.S.	Assistant professor of agronomy, North Carolina College of Agriculture.
V.	Wallace Craig.	Ph.D.	Professor of philosophy, University of Maine.
VI.	Raymond Pearl.	Ph.D.	Biologist, Maine Experiment Station.
VII.	Frank M. Surface.	Ph.D.	Biologist, Kentucky Experiment Station.[1]
VIII.	Maynie R. Curtis.	M.A.	Assistant in biology, Maine Experiment Station.
IX.	Lottie E. McPheters.	—	Computer, Maine Experiment Station.
X.	Frank Pearl.	—	Farmer and practical corn breeder.
XI.	W. Johannsen.	M.D.	Professor of plant physiology, University of Copenhagen.
XII.	P. Boysen Jensen.	Ph.D.	Instructor in plant physiology, University of Copenhagen.
XIII.	Jenny Hempel.	M.Sc.	Assistant in plant physiology, University of Copenhagen.
XIV.	Gerda Dohlmann.	—	Assistant in plant physiology, University of Copenhagen.
XV.	Gilman A. Drew.	Ph.D.	Professor of Biology, University of Maine.

Certain points regarding this list of coöperators need to be discussed. In the first place it is obvious that any one of them (with the possible exception of X.) might in the ordinary course of his work carry out a Mendelian experiment with maize, either independently or in coöperation with someone else. If this were done and the results published they would certainly be accepted by the biological public as a precise and true statement of the facts regarding the material which was in the experimenter's hands. That is, if any worker in this list published a statement that a Mendelian experiment which he had conducted with maize led to a ratio of, for example, 759 : 243 : 252 : 90 this statement would not be doubted or questioned.

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[1] At the time this work was done: Associate Biologist, Maine Experiment Station.

In the second place it is worth while to consider the training, or lines of work with which these 15 observers have had to do. Of six (Nos. I., II., XI., XII., XIII., XIV.) the training and work has been primarily *botanical*. Four of these (the Danish group, Nos. XI. to XIV. inclusive) have had particularly to do with the data of experimental plant breeding, in connection with the brilliant and fundamental researches of Professor Johannsen. The training and special field of work of five (Nos. V., VI., VII., VIII. and XV.) of the observers has been *zoölogical*. Of these five three (Nos. VI., VII. and VIII.) have had experience with the data and methods of investigation in experimental breeding.

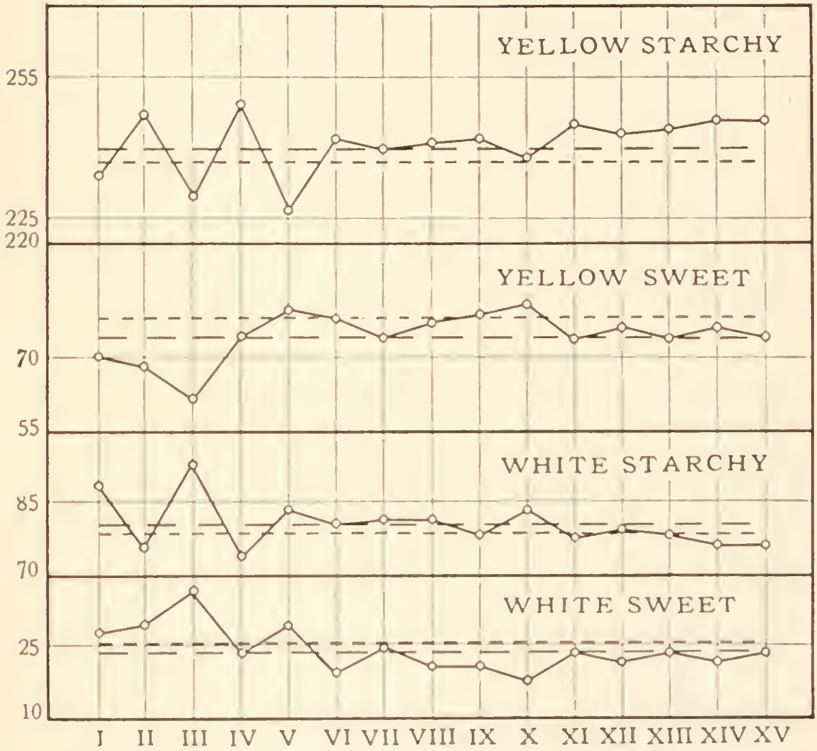

FIG. 2. Diagram showing the count of the different observers of each of the four classes of kernels for ear No. 9.

Table II.

Showing the Classification of the Kernels of Ear No. 8 by the Different Observers.

Observer.	Classes of Kernels.					
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	Total Starchy.	Total Sweet.
Mendelian Expectation.	299.25	99.75	99.75	33.25	399.00	133.00
I.	352	102	52	26	404	128
II.	322	49	82	79	404	128
III.	298	75	108	51	406	126
IV.	332	101	71	28	403	129
V.	305	101	86	40	391	141
VI.	313	100	90	29	403	129
VII.	308	86	95	43	403	129
VIII.	311	101	92	28	403	129
IX.	327	101	78	26	405	127
X.	308	92	95	37	403	129
XI.	311	97	92	32	403	129
XII.	313	99	91	29	404	128
XIII.	308	97	95	32	403	129
XIV.	312	104	91	25	403	129
XV.	333	97	73	29	406	126
Totals.	4.753	1,402	1,291	534	6,044	1,936
Means.	316.87	93.47	86.67	35.60	402.93	129.07

Another of the five (No. V.) adds to the special training of the zoölogist that of the philosopher and psychologist, which by traditional standards, at least, ought to aid in the development of a discriminative judgment. The training of two of the observers (Nos. III. and IV.) has been agricultural. Further, both of these men belong by birth, early life and education to the "corn belt" section of the country, and are thoroughly and intimately familiar with maize. They have had experience in corn judging, which demands the appreciation of very small differences in ear characters. Observer No. X., while not a scientific student of breeding, has had successful practical experience in corn breeding, and is a careful observer. Observer No. IX. has been specially trained in biometric work in the writer's laboratory and has had considerable experience in measuring, sorting small variations out of mixed material, and similar work.

Results.

I.

The results of the counts of the four ears by the different observers are set forth in Tables II. to V. inclusive. Each of

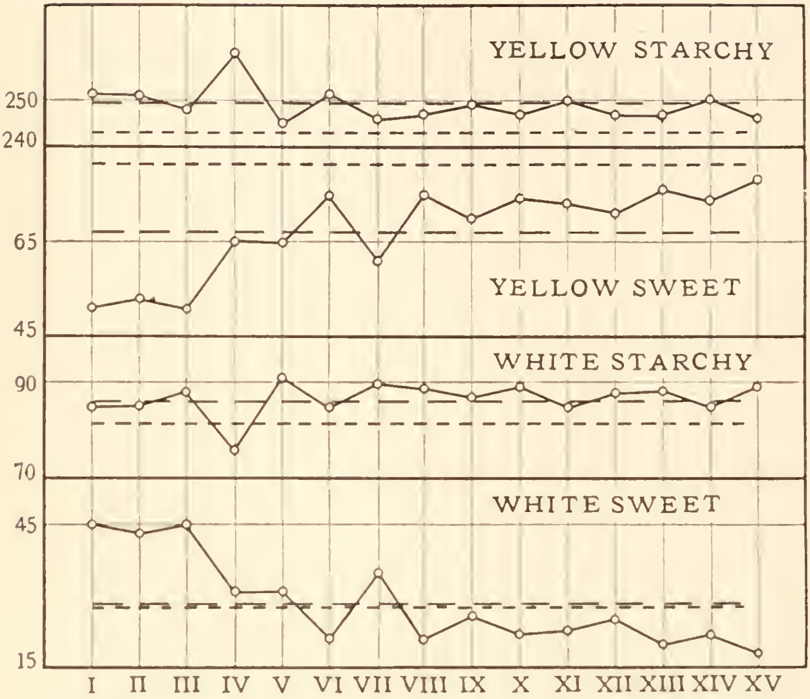

FIG. 3. Diagram showing the count of the different observers of each of the four classes of kernels for ear No. 10.

these tables is arranged as follows: Columns are given for the four different classes of kernels, yellow starchy, yellow sweet, white starchy and white sweet. Also columns are given for total starchy and total sweet. The first row of each table shows the Mendelian expectation for each class. The following lines show the distribution of the kernels as reported by each of the fifteen observers.

The data for the color classes given in these tables are shown graphically in Figs. 1 to 4 inclusive. One of these diagrams is devoted to each of the four ears used in the study. Each figure gives the plotting of each observer's count of the four classes of kernels. The Mendelian expectation is plotted in each case as a dotted straight line and the mean of the results of the different observers as a straight line of dashes.

From these tables and diagrams we note the following points:

TABLE III.

SHOWING THE CLASSIFICATION OF THE KERNELS OF EAR NO. 9 BY THE DIFFERENT OBSERVERS.

Observer.	Classes of Kernels.					
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	Total Starchy.	Total Sweet.
Mendelian Expectation.	237.33	79.11	79.11	26.37	316.44	105.48
I.	234	71	89	28	323	99
II.	247	69	76	30	323	99
III.	230	62	93	37	323	99
IV.	249	75	74	24	323	99
V.	227	81	84	30	311	111
VI.	242	79	81	20	323	99
VII.	240	75	82	25	322	100
VIII.	241	78	82	21	323	99
IX.	242	80	79	21	321	101
X.	238	82	84	18	322	100
XI.	245	75	78	24	323	99
XII.	243	77	80	22	323	99
XIII.	244	75	79	24	323	99
XIV.	246	77	79	22	323	99
XV.	246	75	77	24	323	99
Totals.	3,614	1,131	1,215	370	4,829	1,501
Means.	240.93	75.40	81.00	24.67	321.93	100.07

1. For no one of the ears is there entire agreement among all the observers as to the number of kernels falling in any one of the color classes. There is entire agreement among the observers as to the total number of starchy and sweet kernels in the case of two ears (Nos. 10 and 11), leaving out of account the loss of one starchy kernel from ear No. 10 between the time when this ear was counted by observers X. and XI. In the case of the other two ears (Nos. 8 and 9) there is some disagreement as to the number of starchy and sweet kernels. In no case, however, is the disagreement in regard to these characters so marked as that in respect to color characters.

2. The relative amount of divergence among the observers in regard to the distribution of the kernels in color classes is strikingly different for different ears. Ear No. 9 plainly bore kernels which were relatively easy to classify. The same was true of ear No. 10. On the other hand the kernels of ears 8 and 11 offered many difficulties in classification. But in the case of ear No. 11 the difficulty was largely confined to the sweet kernels, there being close agreement between all the observers but one

TABLE IV.

SHOWING THE CLASSIFICATION OF THE KERNELS OF EAR NO. 10 BY THE DIFFERENT OBSERVERS.

Observer.	Classes of Kernels.					
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	Total Starchy.	Total Sweet.
Mendelian expectation	243.00	81.00	81.00	27.00	324.00	108.00
I.	251	51	85	45	336	96
II.	251	53	85	43	336	96
III.	248	51	88	45	336	96
IV.	260	65	76	31	336	96
V.	245	65	91	31	336	96
VI.	251	75	85	21	336	96
VII.	246	61	90	35	336	96
VIII.	247	75	89	21	336	96
IX.	249	70	87	26	336	96
X.[1]	247	74	89	22	336	96
XI.[1]	250	73	85	23	335	96
XII.[1]	247	71	88	25	335	96
XIII.[1]	247	76	88	20	335	96
XIV.[1]	250	74	85	22	335	96
XV.[1]	246	78	89	18	335	96
Totals.	3,775	1,012	1,300	428	5,035	1,440
Means.	249.00	67.47	86.67	28.53	335.07	96.00

(No. 1) with regard to the yellow and white starchy kernels of this ear.

3. The cause of the discrepancies between the counts of the several observers is obvious from the data. It will be seen at a glance from the diagrams that generally when an observer's count of the *yellow* starchy kernels of an ear, for example, deviated from the mean in excess, this same observer's count of the *white* starchy kernels deviated from the mean in defect, and by an amount approximately corresponding to the positive deviation in the other case. In other words, certain kernels, either starchy or sweet, which were called "yellow" by one observer were called "white" by another. This brings out in a striking way what was obvious to each observer who handled this maize, namely, that there were on each ear a number of both starchy and sweet kernels which were intermediate in respect to color. The distribution of such kernels into the Mendelian categories depends upon the

[1] Between the time when ear No. 10 was counted by observer X. and observer XI. one starchy kernel was lost. Consequently the totals on this ear (sum of all starchy and all sweet kernels) are smaller by one for observers XI. to XV. inclusive than for the other observers.

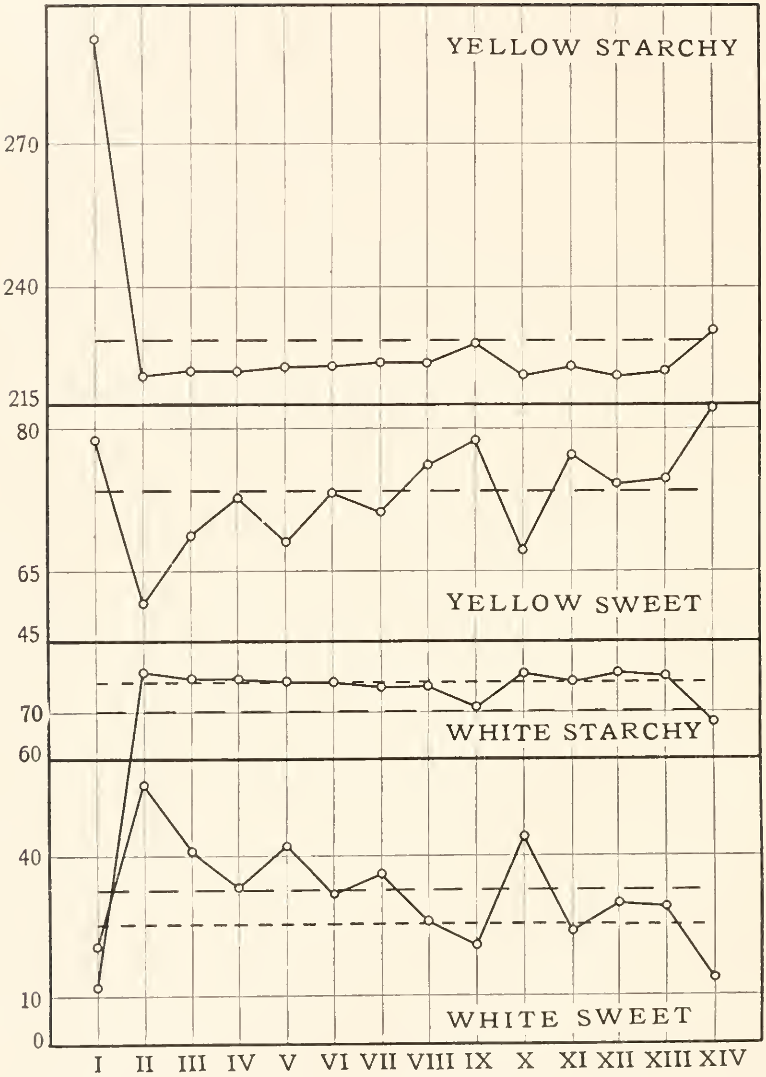

FIG. 4. Diagram showing the count of the different observers of each of the four classes of kernels for ear No. 11.

personal "equation" or bias of each individual observer. As a matter of fact it was possible (and this was done) to make a perfectly graded series of either starchy or sweet kernels from a single ear which ranged from pure white at one end to pure deep

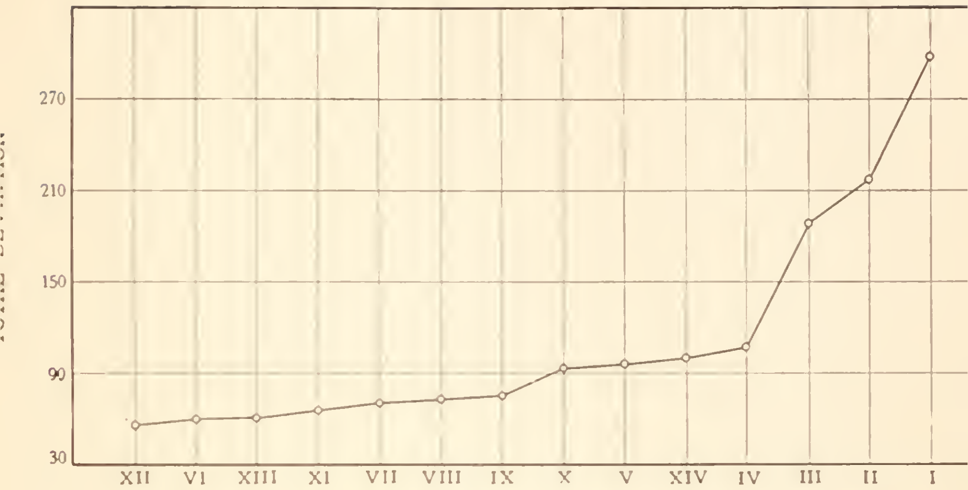

FIG. 5. Diagram showing the total deviations in all the counts of all observers.

TABLE V.

SHOWING THE CLASSIFICATION OF THE KERNELS OF EAR NO. 11 BY THE DIFFERENT OBSERVERS.

Observer.	Classes of Kernels.					
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	Total Starchy.	Total Sweet.
Mendelian Expectation.	[illegible]	[illegible]	[illegible]	[illegible]	305.16	101.72
I.	202	87	7	21	299	108
II.	221	53	78	55	299	108
III.	222	67	77	41	299	108
IV.	222	75	77	33	299	108
V.	223	66	76	42	299	108
VI.	223	76	76	32	299	108
VII.	224	72	75	36	299	108
VIII.	224	82	75	26	299	108
IX.	228	87	71	21	299	108
X.	221	64	78	44	299	108
XI.	223	84	76	24	299	108
XII.	221	78	78	30	299	108
XIII.	222	79	77	29	299	108
XIV.[1]	231	94	68	14	299	108
Totals.	3,197	1,064	989	448	4,186	1,512
Means.	228.36	76.00	70.64	32.00	299.00	108.00

[1] No count of this ear by observer No. XV. is included in this table.

yellow at the other end, with each intermediate step practically as small as one cared to make it. An attempt was made to obtain photographs of such series of kernels which would demonstrate the fact of this gradation pictorially, but the photographic resources at command were not equal to the task and it had to be abandoned.

4. The data presented fully demonstrate, I think, the interesting fact that if each of these fifteen competent, and with one exception (No. X.), specially trained observers had independently undertaken an investigation of Mendelian inheritance in maize, and all used the same seed, of at least the two strains here employed, grown their crops in the same place, and even studied *identically the same* progeny ears, *no two would have fully agreed in the numerical values of the* F_2 *ratios.*

II.

Let us now consider the question as to whether these deviations due to personal equation are of sufficient magnitude to be practically significant. The whole of the remainder of this paper will be devoted to a discussion, from different standpoints, of the quantitative aspects of the recorded classifications of the several observers. All these data will bear upon this general point. To answer the question specifically raised in this section it will only be necessary to show the range of the variation exhibited in the counts made. Table VI. gives for the four ears and the four classes of kernels on each ear (*a*) the mean numbers of kernels found by averaging the counts of all observers, (*b*) the minimum and the maximum recorded number of kernels, (*c*) the total range of variation shown in the records, and (*d*) the percentage which this range is of the mean of the same class.

It is evident from this table that the personal element is one of real significance. When two careful observers can differ in their count of the same set of objects by as much as one and a half times the actual number of the objects counted the factor which leads to this difference is certainly not to be neglected.

An examination of the standard deviations and coefficients of variation of the counts leads to the same result. These constants are shown in Table VII. It should be said in this

TABLE VI.

SHOWING THE RANGE OF VARIATION EXHIBITED BY ALL OBSERVERS IN THE SEVERAL CLASSIFICATIONS.

Ear No.	Class.	Mean.	Lowest Count.	Highest Count.	Range.	Percentage of Range in Mean.
8	Yellow starchy.	316.87	298	352	54	17.0
8	Yellow sweet.	93.47	49	104	55	58.8
8	White starchy.	86.67	52	108	56	64.6
8	White sweet.	35.60	25	79	54	151.7
9	Yellow starchy.	240.93	227	249	22	9.1
9	Yellow sweet.	75.40	62	82	20	26.5
9	White starchy.	81.00	74	93	19	23.4
9	White sweet.	24.67	18	37	19	77.0
10	Yellow starchy.	249.00	245	260	15	6.0
10	Yellow sweet.	67.47	51	78	27	40.0
10	White starchy.	86.67	76	91	15	17.3
10	White sweet.	28.53	18	45	27	94.6
11	Yellow starchy.	228.36	221	292	71	31.1
11	Yellow sweet.	76.00	53	94	41	53.9
11	White starchy.	70.64	7	78	71	100.5
11	White sweet.	32.00	14	55	41	128.1
Mean.						56.2

connection that for the particular sort of problem here dealt with it would appear that the method of expressing the degree of variability which is used in Table VI. (*i. e.*, the absolute value of the range and its relation to the mean) is probably of more real value than are the conventional constants given in Table VII. In the present instance it is the *range* of variation (*i. e.*, the extreme amounts by which different observers differ in their counts) which is the thing of primary interest and practical significance.

Table VII. gives the standard deviation and coefficient of variation for the counts of each class of kernels.

TABLE VII.

SHOWING THE ABSOLUTE AND RELATIVE VARIATION IN THE COUNTS OF THE SEVERAL CLASSES OF KERNELS.[1]

Ear.	Yellow Starchy.		White Starchy.		Yellow Sweet.		White Sweet.	
	Standard Deviation.	Coeff. of Var.	Standard Deviation.	Coeff. of Var.	Standard Deviation.	Coeff. of Var.	Standard Deviation.	Coeff. of Var.
8	13.44	4.24	12.90	14.98	13.85	14.82	13.58	38.14
9	6.09	2.53	4.84	5.98	4.91	6.51	4.69	19.00
10	3.52	1.41	3.46	3.99	9.08	13.46	9.08	31.84
11	17.86	7.82	17.86	25.28	10.52	13.84	10.52	32.88

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[1] Since these constants are not used in any detailed comparisons it has not been thought necessary to calculate probable errors. All the necessary data are at hand, however, if anyone wishes to make these computations. It need only be remembered that for ears 8, 9 and 10, $n = 15$, and for ear 11, $n = 14$.

This table brings out several points which need discussion. These are:

1. The amount of variation, both absolute and relative, in the counts is shown by the measures here used to be very large for some ears and classes of kernels. For no ear, taken as a whole, can the variation fairly be considered negligible. Thus the conclusion previously reached by another method is confirmed.

2. The amount of variation in the sorting and counting is distinctly different for the different ears. From the values of the constants it would appear that ear No. 11 presented the greatest difficulty in respect to the classification of starchy kernels. In respect to sweet kernels ears No. 8 takes rank as offering the greatest difficulties. The starchy kernels of ear No. 10 were the easiest to classify of all starchy kernels. In the case of sweet kernels ear No. 9 had fewer intermediates (*i. e.*, was easier to classify) than any other ear.

3. *Relatively* there was closest agreement among the observers in respect to yellow starchy kernels, and least agreement in respect to white sweet kernels. This table illustrates the fact which was evident to the observers themselves, that there were marked differences in the ease with which the kernels of different ears and different classes could be sorted.

Now while it has been shown that the fifteen observers do not agree in their classification and counts, and that the differences are too large to be neglected, it may fairly be asked if the same result would appear if the group of observers participating were not merely scientifically trained and familiar with maize, but in addition had had a considerable amount of actual experience in the detailed study of variation and inheritance in plants. In other words, is not that special familiarity with the object which comes with the active prosecution of research in a particular field worth something in reducing the magnitude of one's personal error or "equation"? To get some light on this point Table VIII. has been prepared. This is made up in exactly the same way as Table VI., *except* that only observers VI., VII., VIII., IX., XI., XII., XIII. and XIV. are included. These eight observers, comprising the staffs of Professor Johannsen's and the writer's laboratories, have certainly had more extended experience in the direct and immediate study of plant breeding and of variation in plants (involved in all breeding investigation) than have the other observers of the original fifteen. Lists of published papers could be cited in proof of this were it necessary, but the fact is obvious. Will this group of workers on problems of variation and inheritance show a similar degree of variability in their counts to that brought out in Table VI.?

TABLE VIII.

SHOWING THE RANGE OF VARIATION EXHIBITED BY OBSERVERS VI. TO IX. INCLUSIVE, AND XI. TO XIV. INCLUSIVE.

Ear No.	Class.	Mean.	Lowest Count.	Highest Count.	Range.	Percentage of Range in Mean.
8	Yellow starchy.	312.88	308	328	20	6.4
8	Yellow sweet.	98.13	86	104	18	18.3
8	White starchy.	90.50	78	95	17	18.8
8	White sweet.	30.50	25	43	18	59.0
9	Yellow starchy.	242.88	240	246	6	2.4
9	Yellow sweet.	77.00	75	80	5	6.5
9	White starchy.	79.75	77	82	5	6.3
9	White sweet.	22.38	21	25	4	17.9
10	Yellow starchy.	248.38	246	251	5	2.0
10	Yellow sweet.	71.88	61	76	15	20.9
10	White starchy.	87.13	85	90	5	5.7
10	White sweet.	24.13	20	35	15	62.2
11	Yellow starchy.	224.50	221	228	7	3.1
11	Yellow sweet.	81.50	72	87	15	18.4
11	White starchy.	74.50	71	78	7	9.4
11	White sweet.	26.50	21	36	15	56.6
Mean						19.6

It is seen from comparison of this table with Table VI. that the amount of variation in the sorting and counting is distinctly reduced in the group of students of variation. Whereas the average percentage of range in mean is 56.2 for Table VI., it is but 19.6, or only approximately *one third* as much, for Table VIII. Thus it appears that in this case, just as would be expected on general grounds, special experience or practice in a particular line greatly reduces the personal equation. It must be said, however, that even with the group of observers included in Table VIII., the differences are too large to be neglected. When the range of variation amongst different observers of the same thing amounts on the average to approximately one fifth (19.6 per cent.) of the mean value of the thing counted it indicates a source of error not lightly to be dismissed.

III.

It is desirable next to examine somewhat more closely into the nature and distribution of the discrepancies among the observers. A point of particular interest is to determine to what extent the counts indicate a definite and persistent bias on the part of an observer. There may be great variation in the counts of several observers of the same set of things and yet each observer's judgments may be distributed quite at random about the mean. In order to get more light on this and some other matters Table IX. has been prepared. This table gives in successive columns for the four kernel classes, first, the mean deviation from the mean, all deviations being taken together without reference to sign (*i. e.*, the mean total deviation), and second, the mean net deviation from the mean, got by taking the algebraic sum of the deviations. All four ears are used in getting these mean deviations. An example will make clear the method of obtaining the values given in this table. An examination of Tables II. to V. inclusive shows the following set of deviations from the means in the counts of yellow sweet kernels by observer No. V.

+ Deviations from Mean.	— Deviations from Mean.
7.53 (ear 8)	2.47 (ear 10)
5.60 (ear 9)	10.00 (ear 11)
13.13 = sum of + deviations	12.47 = sum of — deviations.

$$\frac{13.13 + 12.47}{4} = 6.40 = \text{mean } \textit{total} \text{ deviation from mean.}$$

$$\frac{13.13 - 12.47}{4} = +0.165 = \text{mean } \textit{net} \text{ deviation from mean.}$$

The last column of the table gives the total deviation from the mean of each observer, all ears being taken together and the deviations summed without regard to sign.

It is strikingly evident from the mean net deviations in this table that each observer was "a law unto himself." Nearly every one of the fifteen evidently had a different system of sorting.

TABLE IX.

SHOWING THE MEAN DEVIATION FROM THE MEAN (TOTAL AND NET) AND TOTAL DEVIATIONS OF THE COUNTS OF ALL OBSERVERS.

Observer.	Yellow Starchy.		Yellow Sweet.		White Starchy.		White Sweet.		Total Deviation on All Ears and All Classes.
	Mean Total Deviation from Mean	Mean Net Deviation from Mean	Mean Total Deviation from Mean.	Mean Net Deviation from Mean	Mean Total Deviation from Mean	Mean Net Deviation from Mean.	Mean Total Deviation from Mean.	Mean Net Deviation from Mean.	
I.	26.93	+23.46	10.10	− 0.34	26.905	−22.905	10.44	+ 0.14	297.82
II.	6.04	+ 2.36	22.09	−22.09	4.68	+ 1.84	21.55	+21.55	217.40
III.	9.29	− 0.29	14.34	−14.34	10.26	−10.26	13.30	+13.30	188.72
IV.	11.04	+ 7.86	2.86	− 0.93	9.93	− 0.75	2.94	− 1.20	107.04
V.	8.79	− 8.79	6.40	+ 0.17	3.34	+ 3.00	5.55	+ 5.55	96.32
VI.	3.54	− 1.08	4.42	+ 4.42	2.50	+ 1.76	4.70	− 4.70	60.98
VII.	4.04	− 4.04	4.50	− 4.50	4.26	+ 4.26	4.55	+ 4.55	70.72
VIII.	3.08	− 3.04	5.92	− 5.92	3.26	+ 3.26	6.20	− 6.20	73.78
IX.	2.89	+ 2.71	6.42	+ 6.42	2.84	− 2.40	6.70	− 6.70	75.38
X.	4.83	− 4.29	6.05	− 0.085	5.26	+ 5.26	6.05	− 0.05	93.52
XI.	4.08	− 1.54	4.37	+ 4.17	3.84	+ 1.51	4.15	− 4.15	66.92
XII.	3.83	− 2.79	3.17	+ 3.17	3.51	+ 3.01	3.70	− 3.70	56.87
XIII.	5.08	+ 0.77	1.87	+ 1.67	4.51	+ 3.51	3.95	− 3.95	61.58
XIV	3.40	+ 0.90	9.17	+ 9.17	3.16	− 0.25	9.45	− 9.45	100.68
XV.[1]	8.07	+ 6.07	4.82	+ 4.55	6.67	− 5.11	5.93	− 5.93	76.46

[1] On three ears only.

Some of these differences are very interesting. For example: No. I. had a high net deviation on starchy kernels, tending to overestimate the yellows and practically zero net deviation on sweet kernels; XIV. is exactly the opposite, having very small net deviations on the starchy and tending strongly to overestimate the yellows among the sweet kernels. No. II. had the tendency to underestimate the yellow sweets, and correspondingly to overestimate the white sweets. No. III. consistently underestimated rather heavily all yellows and overestimated all whites. No. IV. did precisely the opposite. No. V. shows a very erratic set of net distributions, owing to his idiosyncrasy respecting the discrimination between starchiness and non-starchiness. The result is that while he underestimated the yellow starchy kernels, he overestimated all the other classes. No. VI. somewhat underestimated the yellow starchy, but overestimated the yellow sweet. No. X. shows an extraordinarily small net deviation on the sweet kernels, but distinctly underestimated the yellow starchy. In general the table shows in a striking way, that the individuality of the observer is a factor to be reckoned with in work of this sort.

It is of some interest to examine the trend of the total deviations given in the last column. The data are shown graphically in Fig. 5, arranged in order from the smallest to the greatest deviation.

This diagram illustrates a point frequently overlooked. It is commonly argued that the more independent judgments one obtains regarding any point the more accurate will the average result be. We are apt to say that if ten men measure a stick the average of their measurements will necessarily be nearer to the true dimension than if but three men measure and their average be taken. But it is plainly evident from Fig. 5 and Tables VI. and VIII. that the inclusion of observers I., II., III. added nothing to the accuracy of the mean. The point which is forgotten in assuming that greater numbers necessarily mean greater accuracy is apparent if we examine the equation for the probable error of a mean which is

$$P.E._{M} = .67449 \frac{\sigma}{\sqrt{n}}.$$

The probable error, to be sure, varies inversely with n, but it also varies directly with σ, the standard deviation. And, what is here of primary importance, the standard deviation tends to increase as n increases. Whether the probable error shall be smaller or not as the number of observations is increased depends upon what has happened in the meantime to the standard deviation. When n is small, as in the case here under discussion, the effect on the standard deviation of taking $n + 1$ observations as compared with n may greatly outweigh its effect in the denominator of the probable error fraction.

IV.

The next point to be considered is the relative constancy of the same observer's error. If each of the fifteen observers had made a second count of all the ears at some considerable interval of time after the first, how closely would the recounts tally with the original counts? Such an experiment really tests, of course, the stability or constancy of an observer's judgment. It indicates the degree to which his standard of sorting is absolute, and to what extent it fluctuates.

It was not feasible to ask all of the original fifteen observers to go to the labor of recounting these ears. Second counts made after a relatively long lapse of time are, however, available from three observers (namely, VI., VIII. and IX.) for all four ears. While this gives only comparatively meager data, still some points of interest appear. These data are given in Tables X., XI. and XII. It should be said that the recounting was done in the same way as the original count. In each case the observer had no access to the original data while the second count was in progress. No one of the three had any remembrance of what his (or her) original counts were. The writer has not been able to discover any factor which would make these recounts anything other than what they were intended to be, namely, really independent determinations of the same material by the same observers after a long lapse of time.

It will be remembered (*cf.* p. 349 *supra*) that one kernel from ear No. 10 was lost in the course of the original counting. It is therefore obvious that all the recounts of this ear must of necessity be one kernel smaller than the first counts.

TABLE X.

ORIGINAL AND SECOND COUNTS OF EARS 8 TO 11 BY OBSERVER NO. VI.

Ear and Count.	Classes of Kernels.				Date.
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	
8, Original.	313	100	90	29	January 21, 1910.
8, Recount	312	100	91	29	August 10, 1911.
Difference.	−1	0	+1	0	
9, Original.	242	79	81	20	January 21, 1910.
9, Recount.	240	76	83	23	August 11, 1911.
Difference.	−2	−3	+2	+3	
10, Original.	251	75	85	21	January 21, 1910.
10, Recount.	244	73	91	23	August 11, 1911.
Difference.	−7	−2	+6	+2	
11, Original.	223	76	76	32	January 21, 1910.
11, Recount.	223	75	76	33	August 10, 1911.
Difference.	0	−1	0	+1	

The data in these tables indicate that, so far at least as these three observers are concerned, the judgment of the individual is reasonably constant. This is plain if the total deviation of

TABLE XI.

ORIGINAL AND SECOND COUNTS OF EARS 8 TO 11 BY OBSERVER NO. VIII.

Ear and Count.	Classes of Kernels.				Date.
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	
8, Original.	311	101	92	28	January 20,1910.
8, Recount.	309	93	94	36	August 12, 1911.
Difference.	−2	−8	+2	+8	
9, Original.	241	78	82	21	January 20, 1910.
9, Recount.	243	79	80	20	August 12, 1911.
Difference.	+2	+1	−2	−1	
10, Original.	247	75	89	21	January 20, 1910.
10, Recount.	246	73	89	23	August 12, 1911.
Difference.	−1	−2	0	+2	
11, Original.	224	82	75	26	January 20, 1910.
11, Recount.	223	81	76	27	August 12, 1911.
Difference.	−1	−1	+1	+1	

TABLE XII.

ORIGINAL AND SECOND COUNTS OF EARS 8 TO 11 BY OBSERVER NO. IX.

Ear and Count.	Classes of Kernels.				Date.
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	
8, Original.	327	101	78	26	January 21, 1910.
8, Recount.	314	98	89	31	August 11, 1911.
Difference.	−13	−3	+11	+5	
9, Original.	242	80	79	21	January 21, 1910.
9, Recount.	240	76	83	23	August 11, 1911.
Difference.	−2	−4	+4	+2	
10, Original.	249	70	87	26	January 21, 1910.
10, Recount.	246	71	89	25	August 11, 1911.
Difference.	−3	+1	+2	−1	
11, Original.	228	87	71	21	January 21, 1910.
11, Recount.	222	88	77	20	August 12, 1911.
Difference.	−6	+1	+6	−1	

recounts from original counts be considered. In the case of observer No. VI. a total of sixteen kernels were differently classified in the recount from what they were originally. Since the whole number of kernels involved in the experiment was 1,792, this means a discrepancy of less than one per cent. between two independent sortings more than a year apart. Such an error is certainly negligible. Observer No. VIII. classified eighteen kernels, all told, differently in the recount than in the original. This again is only about one per cent. of the total kernels handled, and cannot be regarded as a significant error. In both of these cases (VI. and VIII.) the discrepancies had to do entirely with the color classification. With observer IX. this was not the case. On both ear 8 and ear 9 she classed two kernels as sweet in the recount which she had originally called starchy. Altogether this observer classified thirty-five kernels differently in the recount from what she did in the original. This however represents a relative error of a little less than two per cent. No very great stress could be laid upon such an error.

From the tables it will be noted that there was a marked and nearly uniform tendency on the part of all three observers to underestimate the yellows (both starchy and sweet) and to overestimate the whites, in the recounts as compared with the originals. It seems probable that the cause of this lies, in part at least, in a fading of the yellow color during the time since the first counts were made. Thus it may be that kernels which were plainly yellow when first counted are now white or very nearly so. A further fact which would indicate that fading had occurred is found in the mental impressions of the observers. All three found the material distinctly more difficult to classify when recounted than when originally counted. One feels certain that a part, at least, of this is due to a change in the material itself.

Recognizing fully the meagerness of the material, the facts so far as they go seem to indicate clearly that the same observer is likely to classify the same material in about the same way every time. If a particular kind of bias is shown in one count it will appear essentially unaltered in successive trials. This is probably more true of observers especially experienced in dealing with the data of variation than in the case of those without such experience, though figures are lacking to demonstrate this.

V.

We come next to the consideration of the second question proposed at the beginning (p. 341). This was: "Does somatic 'intermediateness' in maize imply gametic 'intermediateness'? In other words, do F_2 kernels which are intermediate *somatically* give rise to any different sort of progeny when planted than do kernels which belong clearly and indubitably to one or another of the well defined gametic classes in F_2?" To answer this question carefully controlled plantings of somatically intermediate kernels were made in 1910. Series of starchy and of sweet kernels were formed ranging in each case from pure white at one end to pure deep yellow at the other end. Then rows were planted as follows: (1) pure white, (2) deep yellow, (3) the lightest yellow to be found (= somatic intermediates), (4) the yellowest whites to be found (= somatic intermediates). The kernels in classes (3) and (4) were such as would be classified with the yellows by some observers and with the whites by others. The rows included about twenty plants each and were made in duplicate, and in some instances triplicate for both starchy and sweet series. In each row a varying number of ears were self-fertilized (*i. e.*, pollinated by hand with pollen borne on the same plant). Owing to the numerous vicissitudes incident to hand-pollination, together with pressure of other work, as large a number of good ears as would be desirable was not obtained. Some of the possible gametic combinations were not represented at all in the progeny ears. This part of the investigation is, in consequence, not complete. It seems desirable, however, to present briefly the general result shown by the fifty odd ears at hand.

This result was that there was no discernible difference whatever between the progeny of groups (1) and (2) *as a class*, and that of groups (3) and (4) *as a class*. In (3) and (4) some of the kernels planted were of course heterozygotes and some were homozygotes. The same was true, however, of the kernels of (1) and (2). In each case a typical Mendelian result was obtained, and this result could have been predicted in every case (with the exception to be noted presently) had the *gametic* constitution of the kernel been known when it was planted. It could not have been predicted from the *somatic* appearance of the kernel.

The only behavior of an exceptional character observed in these selfed ears was that in certain of the white sweet kernels, which were homozygous recessives in respect to absence of yellowness and starchiness, selfing brought out a latent red.[1] The three ears of this type which were obtained all came from kernels classified in the planting as pure white (group (1)). No such ears were obtained from selfed sweet kernels in group (4). The total number of homozygous, non-yellow sweet ears obtained was too small, however, to make it at all certain that similar red ears might not, with larger numbers, be obtained from group (4) kernels.

It is planned to get further data on this portion of the investigation, using for planting the kernels of ears 8, 9, 10 and 11 which formed the material for the personal equation part of the work. It can be said at this time that the experiments with cross-bred maize so far conducted furnish no evidence that somatic "intermediateness" connotes gametic intermediateness. The progeny of a deep yellow kernel selfed is not visibly different from that of a light yellow kernel selfed, provided both are of the same gametic constitution. The result of this experiment precisely agrees with Darbishire's[2] extensive study of essentially the same problem with peas. Indeed his final conclusion (*loc. cit.*, p. 71) applies here without change of wording: "That in the attempt to predict the result of a given mating the somatic character not only of the parents and of the ancestors of the individuals mated, but of the individuals themselves, may be entirely left out of account; and that the expectation based on a theory of the contents of the germ cells of the two individuals is fulfilled.

Discussion and Summary.

Results such as are set forth in this paper would certainly have been at one time proclaimed by some as furnishing a refutation of Mendelism. In fact one of the earliest criticisms[3] of Mendelian work was mainly devoted to calling attention to the existence of such somatic intermediates between Mendelian categories in the case of peas as are here shown to exist in maize. That such variation, provided it be really somatic or fluctuational, is, however, of no real importance in relation to the cardinal facts of Mendelian inheritance has been shown by all experimentalists who have devoted attention to the matter. Bateson[1] (*loc. cit.*, pp. 240–244) gives an illuminating discussion of the whole matter, with special reference to the phenomena in peas. East and Hayes[2] discuss the same point with reference to maize and show that somatic intermediates behave in inheritance in accord with their gametic constitution rather than their somatic appearance. Certainly the time is past when facts such as are set forth in the present paper can be adduced in criticism of basic Mendelian principles.

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[1] A similar result has recently been described by Emerson, R. A. Rept. Amer. Breeders' Assoc., VI., 233–237, 1911.

[2] Darbishire, A. D., "An Experimental Estimation of the Theory of Ancestral Contributions in Heredity." *Proc. Roy. Soc.*, B, Vol. 81, pp. 61–79, 1909.

[3] Weldon, W. F. R., "Mendel's Laws of Alternative Inheritance in Peas." *Biometrika*, Vol. I., pp. 228–254, Plates I. and II., 1902.

The essential point brought out by this study is, it seems to me, that the well known *general* fact that every datum of science is a function (in the mathematical sense) of two variables, namely, the observer and the thing observed, is once more emphasized by a particular case.

A thorough investigation which brings out essentially this same point, though conducted on a different class of material and with a somewhat different object in view, has been made by Yule.[3]

It will be freely admitted by everyone as an abstract proposition that the personal idiosyncracy of the observer constitutes a source of error in all scientific observing. Yet how often does the biologist not working on strictly quantitative problems make any effort either to eliminate or determine the magnitude of this source of error in his case and in a specific instance? Anyone who has not experimented for himself on the matter can hardly realize how important, on the one hand, and how difficult on the other hand, it is to attain to any considerable degree of real objectivity in results. While the "exact" sciences are somewhat better off in this regard than biology, they are after all not greatly so. There has, to be sure, been a great deal of work done on the theory of errors of observation, particularly as related to astronomy, physics, and like subjects, yet so late as 1902 Pearson[1] demonstrated in a most convincing manner that much of the then currently accepted theory was wrong, and that all of it quite overlooked a factor which might be exceedingly important, namely, correlation of judgments.

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[1] Bateson, W., "Mendel's Principles of Heredity." 2d Edit., Cambridge, 1909.

[2] East, E. M., and Hayes, H. K., "Inheritance in Maize." Conn. Agr. Expt. Stat., Bulletin 167, 1911.

[3] Yule, G. U., "On the Influence of Bias and Personal Equation in Statistics of Ill-defined Qualities." *Jour. Anthropol. Inst.*, Vol. XXXVI., pp. 325–381, 1906.

The present study is by no means a complete investigation of the problem of personal equation in Mendelian work. Correlation of errors ought to be studied, and certain other matters as well. But the present material is statistically entirely inadequate for the discussion of these points, and it does not seem feasible to collect more extensive data, since to do so involves too great a trespass on the time and good nature of busy workers. Further the material here presented brings out clearly the primarily essential points. It shows that in a Mendelian ratio the personal equation of the observer marks a source of error which in the case of maize is of considerable magnitude. This source of error quite overshadows in magnitude, in this case, the error due to random sampling. Yet it is the latter alone which is ordinarily considered by Mendelian workers. The probable error of a Mendelian ratio as commonly calculated tells one the probability that the sample counted is a true representation of the general population from which it was drawn. It tells one nothing whatever about the unconscious bias of the counter as a factor in producing the result set down.

By way of summary it may be said that in this paper evidence is presented which shows that:

1. The observed F_2 Mendelian ratios determined from the same four ears of maize by fifteen competent observers all differ from one another.

2. The failure of all observers to agree in their distribution of kernels into several categories results from two causes, viz., (*a*) the existence of somatically intermediate kernels, and (*b*) the personal bias or idiosyncracy of the observer.

3. The magnitude of the differences between the several observers is such as to demonstrate that the personal equation

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[1] Pearson K., "On the Mathematical Theory of Errors of Judgment, with Special Reference to the Personal Equation." *Phil. Trans. Roy. Soc.*, A. Vol. 198, pp. 235–299. 1902.

is a factor which cannot safely be neglected in work of this character.

4. The observers who have had most experience in the appreciation and measurement of variation have the smallest personal equations on the class of material and the problem here treated.

5. There is no evidence that the progeny of somatically intermediate kernels is different, in any respect whatsoever, from the progeny of distinctly non-intermediate kernels of the same gametic constitution.

DIFFERENTIATION OF THE HUMAN CELLS OF SERTOLI.

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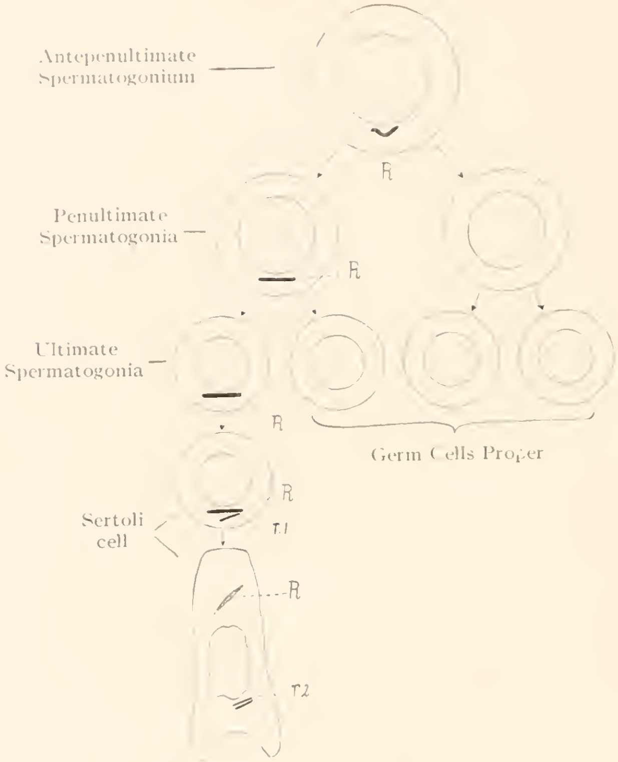
This study is based on the examination of the testis of a negro about 40 years of age, preserved in Zenker's fluid while still warm after his execution. The fixation was not as excellent as might be desired, cytoplasmic details being not always preserved, but the preservation of the nuclei was on the whole very good, and of spindle figures excellent. A considerable variety of staining methods were employed, of which the most fruitful proved to be Heidenhain's iron hæmatoxyline, with various degrees of extraction, followed by alcoholic eosin. Paraffine sections were made of 5μ and 8μ.

For the gift of this material I am indebted to the kindness of Dr. Addison, of the University of Pennsylvania.

1. General Outline of the Process.

The text diagram exhibits the chief results obtained. The antepenultimate spermatogonia contain each a rod (*R.*) within the cytoplasm. This does not divide in mitosis, consequently just half of their daughter cells, the penultimate spermatogonia, come to contain each a rod, while half of them lack it. In the division of these penultimate spermatogonia the rod does not divide but becomes distributed to one quarter of the ultimate spermatogonia. Each of every three ultimate spermatogonia produces by division two primary spermatocytes, and these cells which belong to the true germinal cycle lack the rod entirely. But each fourth ultimate spermatogonium preserves the rod, and this cell without further division enlarges and becomes a cell of Sertoli. In this cell of Sertoli a primary rodlet (*r. 1*) buds off from the rod; then the rod disappears, while the primary rodlet divides into two secondary rodlets (*r. 2*) and the latter persist in the Sertoli cell throughout its history.

The line of the Sertoli cell is therefore determined by the presence of the rod; one Sertoli cell is produced to every three ultimate spermatogonia that lack the rod, or one Sertoli cell to every twenty-five spermatids.


Antepenultimate Spermatogonium
R
Penultimate Spermatogonia
R
Ultimate Spermatogonia
R
Germ Cells Proper
R
Sertoli cell
T1
R
T2

2. The Antepenultimate Spermatogonia (Figs. 1–8, Pl. I.).

These are the largest germ cells in the adult testis, and like the other generations of spermatogonia are situated at the periphery of the seminiferous tubules. Frequently their nuclei are of irregular shape, as shown in Fig. 1. Within the nuclei (Fig. 3) are two kinds of nucleolar structures: acidophilic plasmosomes and basophilic bodies; it would take a detailed study to determine whether the latter are chromatoid nucleoli or modified chromosomes (allosomes). In their cell bodies are found chromatic rods, never more than one to a cell, various forms of which are drawn in Figs. 2–8, that of Fig. 5 being the largest found. The rods are homogeneous in appearance, dense, and they stain with basic stains but usually not as intensely as in the later spermatogonial generations. Such rods are usually in contact with the nuclear surface, but not always, and do not occupy constant positions with regard to the poles of the cell. Characteristic of the antepenultimate spermatogonia is the relatively small size of these rods and their frequently twisted form.

Thirty of these cells were carefully examined, and twenty-three of them showed each one rod. Of the remaining seven, five were not wholly within the plane of the section, so that their rods may have been present in the excised portions. It is probable that each cell of this generation comes to contain a rod, and that the rods are first produced in this generation; no spermatogonia of earlier generations were present, however, consequently there can be no surety of the latter point.

What the method of origin of these rods may be could not be determined. They are quite distinct from the idiozome, at least when fully formed, as shown in Fig. 2. In one case (Fig. 1) no rod was found but an irregular granular mass (x) which is possibly a precursor of the rod; if this be so, the rod might be considered to be produced by the conglomeration of granules at first scattered in the cell body. But the state of fixation of the cytoplasm was not sufficiently reliable to allow of any satisfactory determination of this matter. There is no evidence that the rods are directly produced from the nucleus. Their origin is thus unexplained, though their subsequent history is perfectly clear.

No mitoses of these cells were found, but there can be no doubt that the rods do not become divided for just half of the cells of the next generation contain rods.

3. The Penultimate Spermatogonia (Figs. 9–16).

Forty-nine cells of this generation were examined, care being taken to study only those that lay entirely within the plane of the section, and of these twenty-four exhibited each a rod while twenty-five showed no rods. Also six cases were found of two nuclei in one cell body, indicating nuclear division without cytoplasmic division of antepenultimate spermatogonia: in all six of these cases only one rod was present, and an example is shown in Fig. 9. There can thus be no doubt that half the penultimate spermatogonia contain rods and half do not.

Characteristic appearances of the rods are illustrated in Figs. 9–11, Pl. I., 14–16, Pl. II. They differ on the average from those of the preceding generation in being usually larger, straighter, and more deeply-staining with hæmatoxyline, which indicates they have been undergoing growth changes.[1] Not infrequently they are curved around the nuclear surface (Fig. 14) and the length of a rod may equal the diameter of a nucleus. Consequently they are in this stage very prominent constituents of the cell bodies and easily differentiated by safranine or hæmatoxyline.

Mitoses of these cells were not frequent, but two clear cases (Pl. II., Figs. 12, 13) were found, showing that the rod (*R.*) passes undivided into one of the daughter cells, and this is fully borne out by a study of their distribution in cells of the following generation. Fig. 18 shows the end result of such a mitosis in a case where the cell body had not divided and here there is but one rod. What is the nature of the scattered globules shown in Figs. 12 and 13 is doubtful; they may be discharged nucleolar material.

4. THE ULTIMATE SPERMATOGONIA (FIGS. 17–24, PL. II.).

These are the smallest of the spermatogonia and the most numerous in the testis studied. One quarter of them contain each a rod; three quarters lack rods. One hundred and forty-two of these cells were studied, at stages before any of them had enlarged into Sertoli cells, the precaution being taken to include only cells lying wholly within the section; of these twenty-five showed each one rod, and one hundred and seventeen showed no rods. This ratio is somewhat less than 1 : 3, which is readily explained on the ground that some of the spermatogonia with rods had already become Sertoli cells and therefore were not included in the count. A very important and clear case is that of Fig. 17; this shows four nuclei, the granddaughters of the nucleus of an antepenultimate spermatogonium, while there has been no division of the cell body, and it will be seen there is but one rod to the four nuclei. The evidence is then decisive that one quarter of the cells for this generation contain each one rod.[1] In these cells the rods are on the average more massive than in preceding generations (Figs. 17–24), and while usually more or less curved are never twisted. Quite frequently one end of the rod is bent off at an angle (Figs. 17, 22). In these cells also the rods are most dense and acquire their maximum stain, staining fully as intensely as the basichromatin; they generally but not always touch the nuclear surface.

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[1] The condition of the pair of rodlets (*r. 2*) in Fig. 10 will be explained later.

5. Differentiation and History of the Sertoli Cells (Figs. 25–50).

All the ultimate spermatogonia that contain rods become cells of Sertoli, and those only. Nothing like either rods or rodlets were found in any of the spermatocytes or spermatids. The Sertoli cells become especially marked by their great growth. Fig. 25, Pl. II., shows the beginning of such growth, the Sertoli cell (*A*) growing out beyond its sister ultimate spermatogonia (*B* and *C*). Figs. 34 and 35, Pl. III., show Sertoli cells in a later growth stage in their entirety, and Figs. 36–39, 41–46 exhibit portions of them in still later stages. These cells became relatively enormous as shown in Fig. 50, Pl. V., which represents a portion of a transection of the wall of a seminiferous tubule; in this figure the shaded portion represents the bodies of the Sertoli cells, which have grown far into the lumen of the tubule to embrace the spermatids. This great growth is due mainly to the formation of vacuoles within the cytoplasm, and in the figures only the larger of the vacuoles are shown, not the great number of minute ones. These vacuoles are drops of a non-staining fluid, like that contained within the cavity of the tubule; only in rare instances are any concretions found in the vacuoles. One end, the basal, of each Sertoli cell remains adherent to the fibrous wall of the tubule and in the figures lines are drawn to denote the inner border of the tubule; the other end, the distal, is the one that grows out and forms branches ramifying around the spermatocytes and spermatids. In the later history of the Sertoli cells large spaces are found within them, as shown in Fig. 50, which are cavities in which germ cells had been situated before their transformation into spermatozoa. Boundaries between the Sertoli cells become indistinguishable, so that these cells come to constitute a syncytial cytoplasmic net of extremely vacuolar structure (Fig. 50). In the basal portions of the Sertoli cells parallel bundles of fibrils may be seen at certain stages (Fig. 43).

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[1] Spermatogonia with two, three or four nuclei in a single cell body are unusually frequent and in such cases the sister nuclei are frequently of quite unequal volumes.

The nuclear changes are also characteristic, and represent a gradual transformation of the structure of the resting nucleus of an ultimate spermatogonium. The reticulum changes first into microsomal masses (Fig. 25, Pl. II.). Then takes place a flowing of these masses together (Figs. 34–39, 41–43, Pl. III., IV.) until all the basichromatic substance of the nucleus becomes concentrated into a mass or karyosphere, and the particles remaining without the mass are oxychromatic. Figs. 44 and 45, Pl. V., represent the result of this process. Then follow stages of dissolution of the karyosphere into minute granules, all of which become gradually oxyphilic (Figs. 46 and 48), Fig. 49 representing a degenerate nucleus at the close of the cell's cycle. During all these stages the nuclei become very irregular, with deep indentations and lobations at their margins and grooves passing along their lengths. This irregularity of form and the central karyosphere are diagnostics by which these nuclei may be readily distinguished from those of neighboring germ cells. Further, the nuclei do not remain at the basal end of the cell, as they do in certain other mammals, but move out beyond the level of the spermatogonia (Fig. 50).

After passing through the series of changes just described the Sertoli cells degenerate, for there is no evidence that they go through a second cycle. This is proven by the later stages of these nuclei (Figs. 47–49) which gradually become wholly achromatic and then disappear from view. Their vacuolar substance must at that time mingle with the fluid of the tubule. Fig. 25 (Pl. II.) is interesting in this regard, for it exhibits a young Sertoli cell (*A*) pushing out before it an old and degenerate one (*D*).

A Sertoli cell is therefore produced to every twenty-four spermatids, and after the latter have metamorphosed into spermatozoa and these spermatozoa have become discharged from the tubules, that Sertoli cell degenerates. Formation of Sertoli cells must then continue through life as long as formation of germ cells continues.

We now pass to the history of the rod in the Sertoli cells, that remarkable body which differentiates them from the functional germ cells. This is at first a simple rod, and may remain such even in the beginning enlargement of the cell (Fig. 25, Pl. II.). But this rod divides, and in most cases before the Sertoli cell begins its growth. Stages of its division are rarely found, and the only ones observed are illustrated in Figs. 26–30, Pl. III. It will be seen that in the cases of Figs. 26–28 the rod is undergoing an unequal longitudinal cleavage, a more slender and shorter rod abstricting from a portion of the larger one; this smaller rod may be called the primary rodlet. Perhaps one reason why these stages are so seldom found is because this division can be seen clearly only when the rod lies at a particular angle of vision. Whether Fig. 30 represents simply an unusually bent rod, or one that is in process of division, is hard to determine, for it was an isolated case. The condition immediately following this division is shown in Fig. 31, with an unusually long primary rodlet (*r. 1*) completely separated from the rod (*R.*); this is a cell body of the volume of that of an antepenultimate spermatogonium, where accordingly cytoplasmic division had not occurred, and where the original nucleus had divided while only one of its daughter nuclei had divided again. A case of rod and primary rodlet together at an unusually late stage is represented in Fig. 41, Pl. IV.

In four fifths of the cases, in 81 out of 100 cells examined, the large rod completely disappears before the Sertoli cell starts in its growth and in such cases only the primary rodlet is to be seen (Figs. 32–33, Pl. III.). Just so soon as the cell enlarges a pair of secondary rodlets are seen instead of the primary rodlet (Fig. 34), and without doubt these are produced by equal longitudinal cleavage of the primary rodlet, for they are always of the same length and lie close together. In their later stages (Figs. 43, 46, 47) these secondary rodlets undergo some increase in thickening, and in all cases these rodlets persist within the Sertoli cell until the end of its cycle; they also probably degenerate there, for no signs of them were found within the germ cells or free in the fluid of the seminiferous tubule.

But in one fifth of the cases, 19 out of 100 cells examined, the original rod continues visible for a shorter or longer period after the secondary rodlets have been produced, as shown in Figs. 35–39, 42, 45; and in Fig. 41 is drawn an unusual case of late persistence of the rod and primary rodlet together. The rod may persist for a while as a single dense body (Fig. 38). Fig. 39 shows a case of such a single rod that has segregated into chromatic and achromatic parts, a rare condition. But as a rule it divides longitudinally as exhibited in Figs. 35–37, 41, 42, 45; this division begins and is most prominent near the middle region of the rod, when its ends may be still undivided, but cases were found where the rod had completely divided into two secondary rods (Figs. 35, 40, Fig. 40 being a rod from a cell of about the stage of the cell shown in Fig. 39). In the instances where the rod persists after the secondary rodlets have been produced, it never stains quite as deeply as the latter, and gradually becomes less and less chromatic until it can no longer be seen; no rod was observed in any cell after the karyosphere of the nucleus had disintegrated.

There is accordingly considerable individual variation in the behavior of the rod after it has abstricted the primary rodlet; in four fifths of the cells it then promptly disappears, in one fifth it persists for a variable period, but never until the end of the cycle of the Sertoli cell, and then undergoes a second longitudinal division which is this time an equal division. The rod when it persists generally remains in the basal portion of the cell body. The secondary rodlets are at first usually in contact with the surface of the nucleus, either basal or distal, while they are later found near the distal pole of the nucleus and usually separated from it. Whether the disappearing rod contributes substance to the formation of the fibers in the cytoplasm (Fig. 43) could not be determined. It is also difficult to decide whether the rods and secondary rodlets are or are not always enclosed in vacuoles.

Certain aberrant cases need mention. In a single instance a pair of secondary rodlets were found together with a rod in a penultimate spermatogonium (Fig. 10), a precocious case of rodlet formation; what the constricted acidophilic body in the cytoplasm of this cell may be, I do not know. Then in each of two cases of rather late Sertoli cells, instead of the general case of one pair of secondary rodlets, two pairs were found (Figs. 44, 48); these might have been produced from an unusually long primary rodlet, such as the one shown in Fig. 31, by the occurrence of a transverse as well as a longitudinal division.

6. Discussion and Conclusion.

It is truly surprising that no thorough account has yet been given of the human cells of Sertoli; indeed, the studies made so far are rather histological than cytological.

Attention to these cells was first drawn by Sertoli (1865), who called them "cellule ramificati." Most writers since his time have given them his name; but the term "follicle cell" (coined by Valette St. George) is frequently employed, as well as the term "foot cell" (J. E. S. Moore), while v. Ebner ('71) employed the name spermatoblast, and Benda ('94) that of vegetative cell. The name follicle cell is generally used for the cells composing the spermatocysts of invertebrates and lower vertebrates, and that of Sertoli cell for the physiologically correspondent cells of mammaliam testes.

As to the genetic relations of the Sertoli cells to the germ cells proper the writers fall into groups, which Waldeyer ('06) has designated as the dualists and the monists. The first of these regard the two kinds of cells as of entirely different origin, the spermatogonia proceeding from primordial germ cells and the cells of Sertoli from other elements. As dualists are to be classed Watase, Bardeleben, Benda, Waldeyer and Stephan. Watase ('92) and Bardeleben ('97) consider the Sertoli cells to be interstitial testis cells that have wandered into the seminiferous tubules; but Bardeleben's figures are quite indecisive, and Watase, in his very brief account of little over a page, drew his conclusions from the similarity in color of the cells of Sertoli and the interstitial cells after staining with cyanine, chromotrop and erythrosine. Though Bardeleben thus holds the two kinds of cells to be of different origin, he nevertheless thinks that the Sertoli cells give rise to "a rudimentary second form of spermatozomes." Benda ('94, '98), and Waldeyer relying upon him, considers the cell of Sertoli to arise from the indifferent cylindrical peritoneal cells. The dualists generally hold that a differentiated Sertoli cell remains functionally active during the life of the individual and does not regenerate more than the distal portion of its cell body; and they are also of the opinion that the cells of Sertoli proliferate themselves by division—amitotically, according to Bardeleben and Stephan, or mitotically according to Benda. On the other hand Prenant ('87), Schoenfeld ('01), Regaud ('99) and Bugnion ('06) consider both cells of Sertoli and spermatogonia to be derived from one kind of cells, by a process of division of labor; and this is in agreement with the results of most writers who have studied the origin of the follicular cells of the ovaries and testes of invertebrates—the follicular or nurse cell being generally regarded as a modified germ cell. Yet Regaud and Stephan ('02) hold that the fully formed Sertoli cells proliferate germ cells as well as nourish them; while Bugnion believes "the primordial spermatogonium gives place to a plurinucleate plate (part of the parietal synctium) which contains in a common cytoplasm spermatic nuclei and sertolian nuclei," after which the germ cells delimit themselves from the syncytium that remains as a Sertoli cell.

My conclusions differ practically in their entirety from those of the writers mentioned. In the human testis the cells of Sertoli are of common origin with the germ cells, one out of every four ultimate spermatogonia becoming a Sertoli cell. Sertoli cells are thus not differentiated from the germ cells merely in early fœtal history, but so long as ultimate spermatogonia continue to be produced. A Sertoli cell of man once differentiated does not, so far as I have observed, divide again, and consequently does not give rise to germ cells; further, a Sertoli cell dies completely after the spermatozoa that are associated with it depart from its surface, and it does not persist to nourish a second generation of spermatozoa. There being one Sertoli cell to every three definitive ultimate spermatogonia there is necessarily one to every twenty-four spermatozoa; accordingly, in man the number of spermatozoa, spermatic bundle, associated with one Sertoli cell cannot be "8 or 16" as Bugnion states.

But the point of the greatest interest with regard to the differentiation of the human Sertoli cell, is that it is determined by the inclusion of a peculiar cytoplasmic rod, this rod first arising in the antepenultimate spermatogonia. No such "Sertoli cell determinant" has been made known in any other object. In the case of the differentiation of the oögonia from the nurse cells in the ovary of the beetle *Dytiscus*, so well described by Giardina ('01), and corroborated by Debaisieux ('09), there is a remarkable mechanism of differentiation of the nurse cells: here the cells that are to become oöcytes receive a cast-off reticular part of the nucleus, while the cells which lack this extruded mass become nurse cells. It will be seen that this is an entirely-different process from that described by me for man, for in man the Sertoli cells are those that contain the differentiating body.

The development of the human Sertoli cell is clearly a very beautiful case of somatic differentiation. In fact, one may regard the multicellular organism as having two periods of somatic differentiation: the first when the tissue cells become differentiated from the germ cells, and the second, when in the early mass of germ cells, the primordial gonad, the Sertoli cells become differentiated from the germ cells proper. For the Sertoli cells may properly be classed as the soma of the testis.

Nothing like the rod that differentiates the human Sertoli cells from the other ultimate spermatogonia seems to be known in any other case of somatic differentiation. In the classical case of *Ascaris*, discovered by Boveri, prospective body cells cast off into the cytoplasm the ends of their chromosomes. In copepods and insects, according to Häcker and Silvestri respectively, a nucleolus or a mass of nucleolar substance thrown out from the germinal vesicle of the egg comes ultimately to lie in cells of the germinal cycle. The origin of the rod of the human Sertoli cells I could not determine, beyond that it is first apparent in the cytoplasm of the antepenultimate spermatogonia, and that it probably forms there during the rest stage of the cells. It comes to develop in all antepenultimate spermatogonia, therefore, before the distinction of Sertoli cells and germ cells; it becomes transmitted without division to one quarter of the ultimate spermatogonia, and that quarter transforms into Sertoli cells. Under these conditions, on account of the precision of the process, this rod must be regarded as a Sertoli determinant, and as a cytoplasmic and not a nuclear determinant. Whether the rod, or its substance, emanated in the first place from the nucleus, can be determined only by some fortunate observer who has more and better fixed material than was in my hands. But there is no reason to regard it as mitochondrial, as a chondriosome, because granular mitochondria have been described in mammalian Sertoli cells by Benda and others; in my material no mitochondria were seen in the spermatocytes and spermatids, they were evidently dissolved by the action of the fluid of Zenker, and it is therefore probable they were dissolved also out of the spermatogonia.

The rod that comes to determine the Sertoli cells increases in size while in the cytoplasm, becoming most voluminous in the ultimate spermatogonia; outside of the nucleus, also, occurs its process of abstriction of the primary rodlet and the division of the latter into the secondary rodlets. It is therefore clearly an extranuclear determinant of the Sertoli cell; and this as yet unique process of somatic differentiation seems to be controlled by an extranuclear body.

It has not been my intention to decide upon the function of the Sertoli cells. They increase greatly in size to produce a syncytial mass loaded with intracellular droplets, probably of fatty nature; they envelope closely the rapidly growing spermatocytes and for this reason they are generally supposed, and probably correctly so, to nourish this generation of germ cells. The fluid within the seminiferous tubules contains, so far as I have observed, neither erythrocytes nor leucocytes, therefore is probably derived from the droplets of the Sertoli cells and not from the blood serum. The spermatids at the commencement of their histogenesis lose their first connection with the Sertoli cells, while the nearly mature spermatozoa exhibit their heads buried in the substance of the Sertoli cells; the latter is then a second orientation of the germ cells to the Sertoli cells, one that cannot subserve nutrition, for the developing spermatozoa do not increase in size, but which is rather, as Loisel ('07) has shown, the expression of some chemico-tactile response. It may then be the Sertoli cells fulfill three functions: to nourish the spermatocytes, to furnish the fluid within the seminiferous tubules, and to attract the spermatozoa into oriented bundles.

It is certain that much more study is needed of the Sertoli cells, both from the standpoint of somatic differentiation as well as that of the physiology of the germ cells themselves.

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EXPLANATION OF PLATES I–V

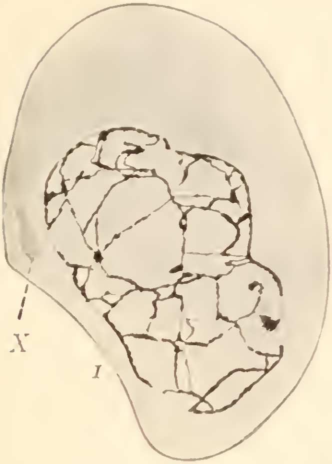
All figures have been drawn by the author with the camera lucida at the level of the base of the microscope, and reduced one third in size in reproduction. Fig. 50 was drawn with Zeiss obj. C, ocular 12, all the others with the apochromatic immersion objective 1.5 mm., ocular 12.

The following abbreviations have been employed:

Id., idiozome.
R., rod.
r. 1, primary rodlet.
r. 2, secondary rodlets.
S.C., Sertoli cells.
Sp.G., ultimate spermatogonia.

PLATE I.

FIGS. 1–3. Entire antepenultimate spermatogonia.
FIGS. 4–8. Rods of antepenultimate spermatogonia.
FIG. 9. A binucleate penultimate spermatogonium.
FIGS. 10, 11. Penultimate spermatogonia.



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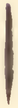
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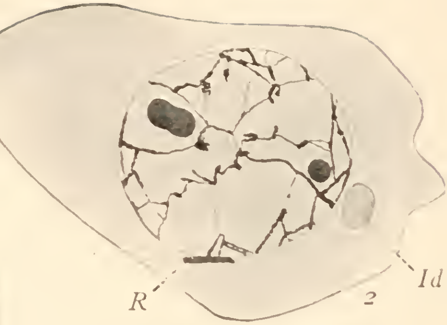

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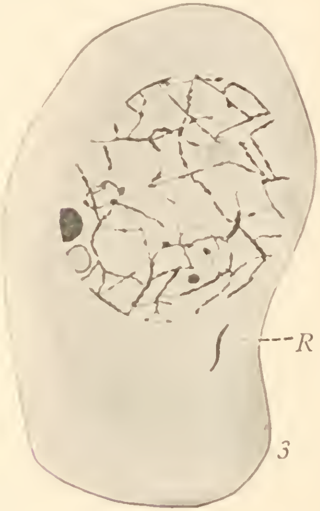

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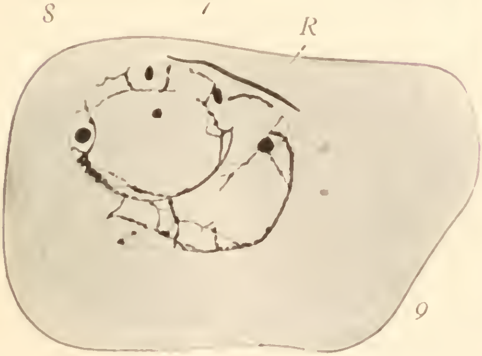

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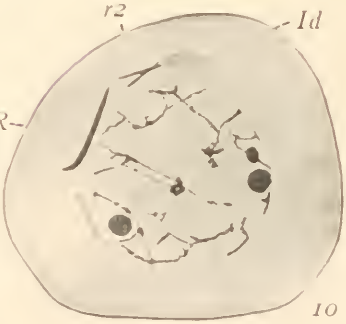

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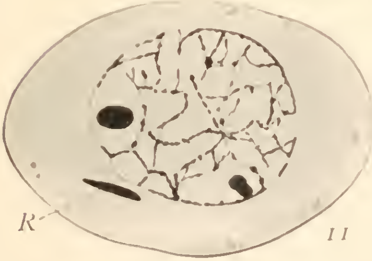

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PLATE II.

FIGS. 12, 13. Penultimate spermatogonia in division.

FIGS. 14–16. Rods of penultimate spermatogonia.

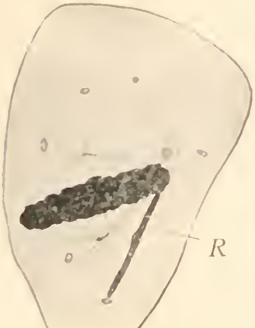
FIG. 17. Quadrinucleate ultimate spermatogonium.

FIG. 18. Binucleate ultimate spermatogonium.

FIGS. 19, 20. Ultimate spermatogonia.

FIGS. 21–24. Rods of ultimate spermatogonia.

FIG. 25. An incipient Sertoli cell (*A*) next to two ultimate spermatogonia (*B*, *C*), and to a degenerate Sertoli cell (*D*). The inner margin of the wall of the seminiferous tubule is at the left.



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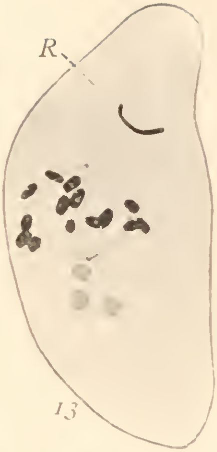

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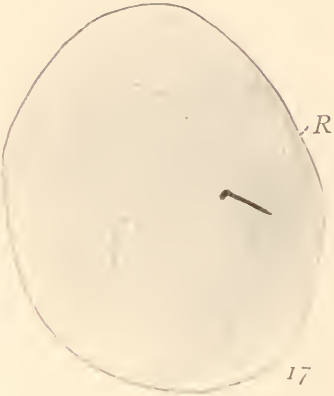

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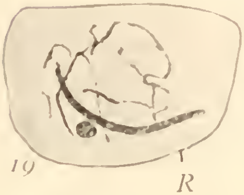

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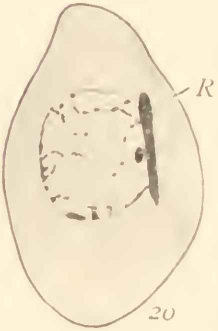

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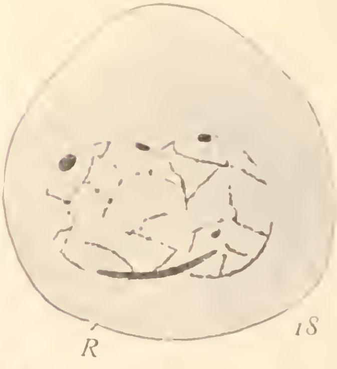

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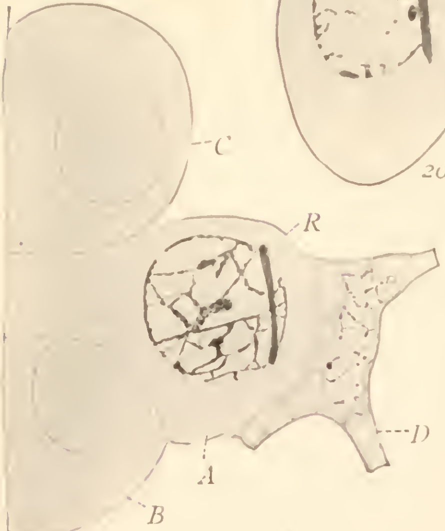

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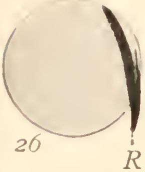
PLATE III.

FIGS. 26–30. Primary rodlet abstricting from the rod, early Sertoli cells.
FIG. 31. Trinucleate ultimate spermatogonium.
FIGS 32, 33. Early Sertoli cells with primary rodlets.
FIGS. 34–36. Secondary stages of Sertoli cells.



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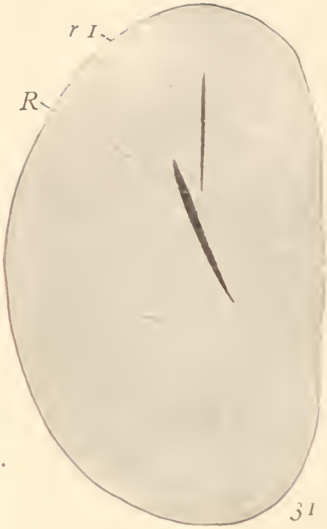

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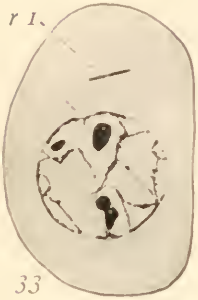

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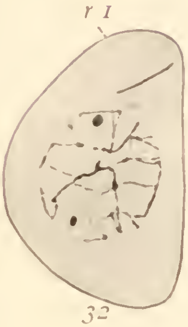

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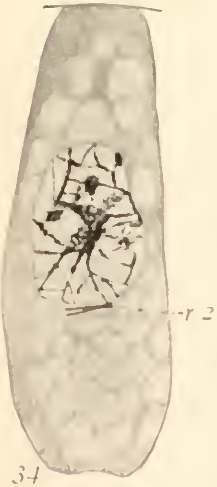

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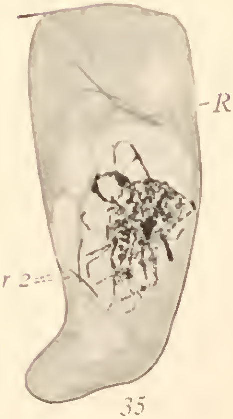

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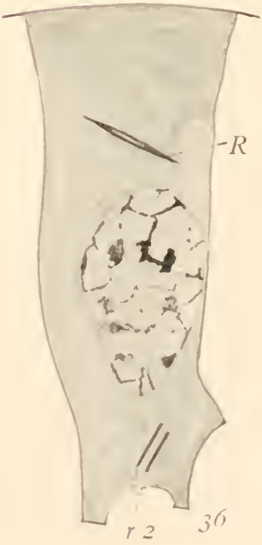

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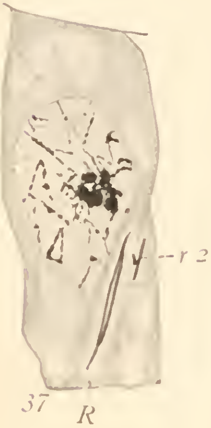
PLATE IV.

Succeeding stages of Sertoli cells arranged in the order of the nuclear changes Fig. 40 exhibits a dividing rod of a stage similar to that of 41.



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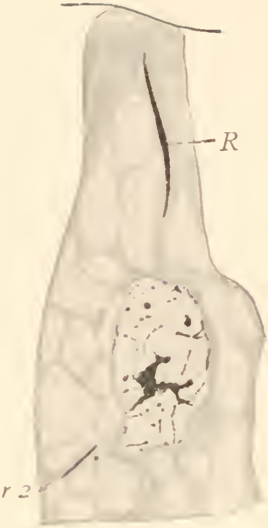

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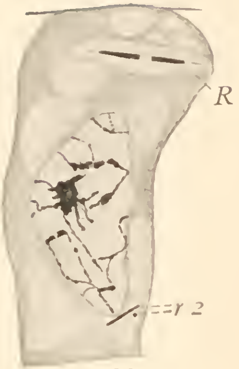

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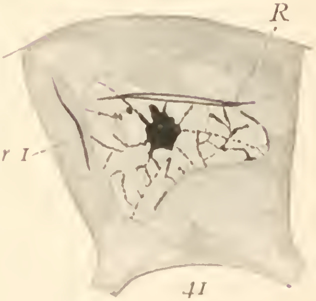

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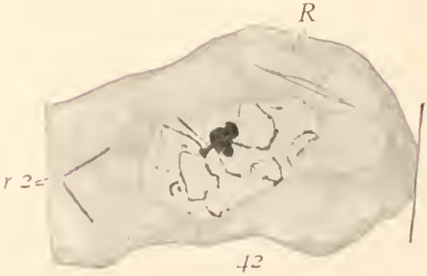

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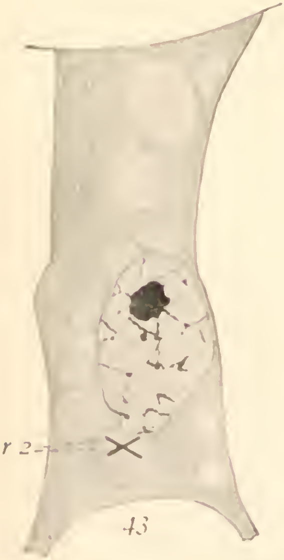

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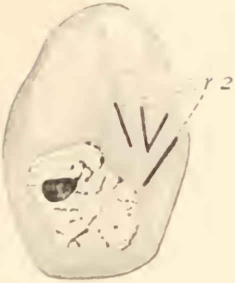
PLATE V.

FIGS. 44-47. Later stages of Sertoli cells, Figs, 44 and 47 being oblique transsections.

FIGS. 48, 49. Degenerate nuclei of late Sertoli cells.

FIG. 50. Portion of a section of a seminiferous tubule. Uppermost is the wall of the tubule, and next to it a layer of ultimate spermatogonia. The syncytium of the Sertoli cells is expressed by dark shading, and their nuclei are distinguishable by their angular form and central chromatic body. The other cells shown are chiefly early spermatids.



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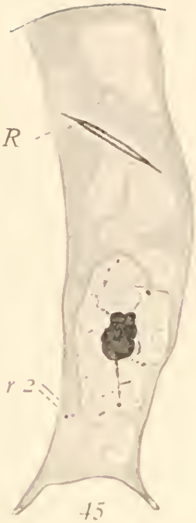

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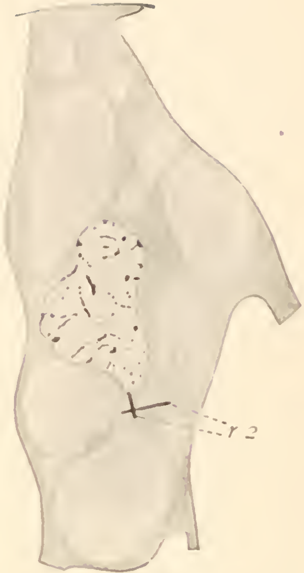

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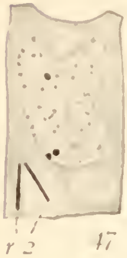

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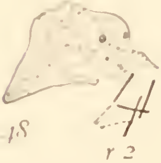

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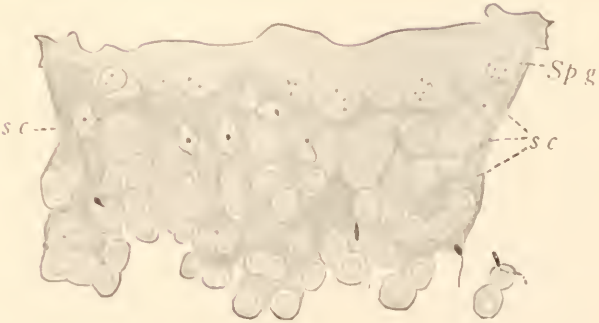

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T. H. MONTGOMERY.

ON THE CAUSE OF AUTOTOMY IN TUBULARIA.

OSCAR RIDDLE.

In the course of studies on the oxidizing and reducing powers of the various tissues and body regions of hydromedusæ the phenomenon of autotomy was observed to occur so generally and so rapidly in *Tubularia* as to invite attention to its cause. That *Tubularia* is capable of autotomy—*i. e.*, of the self-division of its body—has long been known, the process having been observed by Giard, Loeb, Driesch, Morgan, and others. Two investigators have definitely sought to determine the *cause* of the phenomenon. The conclusions of these two workers seem, however, not to be in accord. Godlewski[1] maintains that degeneration of the hydranth precedes and conditions the autotomy, so that it is a *degenerated* hydranth that is severed from the body. Morse[2]—who seems unfortunately to have overlooked Godlewski's paper—writing in this journal states that autotomy may occur without an initial degeneration in the hydranth, if one can judge of this from histological examination.

My own experience with autotomizing *Tubularia* indicates that when this process is effected very slowly and gradually, as is ordinarily the case, one can certainly sometimes find, as did Godlewski, that before the actual separation of the hydranth the latter has undergone considerable degeneration. On the other hand, I have had many autotomized hydranths to live in apparently perfect condition for three and four days. Godlewski himself notes one such hydranth which lived for two days. It is quite easy, too, to confirm Morse's statement that a rise in temperature favors the occurrence of autotomy. Nevertheless, the peculiar conditions under which I was able to observe the autotomy convinced me that neither of the above mentioned supposed causes, nor yet both combined, is the immediate or adequate cause of autotomy. I therefore gave a little special attention to this subject, the results of which may be summarized here.

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[1] Godlewski, E., "Zur Kenntnis der Regulationsvorgange bei *Tubularia mesembryanthemum*." *Roux's Archiv*, Vol. 18, 1904.

[2] Morse, Max, "The Autotomy of the Hydranth of *Tubularia*." BIOLOGICAL BULLETIN, Vol. 16, 1909.

The particular experience which seemed to contravene the proposed causes as being the actual immediate cause was the following: If normal healthy individuals of *T. mesembryanthemum* be held by the lower stem or stolon and drawn through any one of a variety of solutions—sodium tellurite, sodium selenite, etc.—*complete autotomy may occur in less than one minute!* Clearly degeneration is not the cause of the autotomy in these cases. Other members of the colony taken from their moorings and placed in vessels supplied with fresh sea water remained for days without autotomy. When some of these were similarly drawn through pure sea water they remained intact without autotomy. It was found, moreover, that the autotomy likewise occurred even when the animal was dipped into a solution of Na_2TeO_3, $NaSeO_3$, etc., of *lower* temperature than that from which it had just been removed. Here, too, the autotomy was rapidly and decisively effected. In these cases the autotomy plainly could not have been caused by a rise in temperature; the temperature change actually being in the opposite direction. In many cases the animals were removed from water at 16° C. and drawn through a solution at 13° C.

In order to study the changes occurring in the rapidly autotomizing animals these were examined with a Zeiss binocular while being drawn through the solutions. By this means it was found: (1) that as soon as the animal touches the solution there follows a very *strong contraction* of tentacles, hypostome, peristome, etc.; (2) that the "neck" region becomes *extremely contracted* and narrow, and apparently so much weakened as to be unable longer to support the weight of the hydranth; or rather too weak to sustain the slight pull on the hydranth as it is being drawn through or lifted from the solution. The appearance here is such as to indicate that the contraction of the circular fibers of this region is of sufficient force, not only to close completely the central channel, but also to separate and crowd out many entoderm cells, and likewise to weaken their own adhesion and that of the other ectoderm cells to each other. *A vigorous con-*

traction in response to stimulation therefore seems to be the effective cause of autotomy.

It has been possible in a few instances to get a beautiful demonstration of the strength of the contraction in the "neck" region. If a tubularian be found with the gastro-vascular cavity of the hydranth well expanded and full, it can sometimes be induced—by pricking the peristome—to contract the peristome first, and thus retain the whole of the fluid of the cavity. In this condition the hydranth somewhat resembles a rubber ball; the channel to the outside being closed by the contracted peristome, and the posterior continuation with the cavity of the stem or body being interrupted by the above-mentioned contraction of the "neck" region. If now, with an appropriate blunt instrument, pressure be brought to bear upon this sphere, and the whole proceeding carried out under the binocular, one can watch everything and gauge with one's own muscles the strength of the contraction in the peristome and "neck." In the instances where I have carried out this experiment *I have never been able to force the opening of the channel in the neck region.* Some part of the hypostome wall is the first to break.

It is, too, this very strong contraction that carries the cœnosarc of the *neck region* quite away from the perisarc (see Fig. 0). Probably the reason that the point of the autotomy is always so definitely localized in this "neck" region—as was first recognized by Giard[1]—is that the remainder of the slender portion of the animal is covered with a chitinous perisarc which is rather impermeable and highly protective against stimuli.

In "normal" cases—those in which the process of autotomy is extended over a period of several hours or a day or two—it is well known that a very complete histolysis of the cells in the "neck" region occurs. There is good evidence however that in these cases, too, the histolysis is preceded by a rather strong or by a prolonged contraction of this region. In very weak solutions of tellurium and selenium salts, of acids and alkalis, and even by watchful mechanical stimulation of the animal into continuously contracted state, I have been able to effect the autotomy of healthy hydranths more gradually in periods extended to one to four hours, and to watch the course of the process in a single individual during this time. From these observations I may here record one or two points which seem to throw some light on the reason for the histolysis just mentioned.

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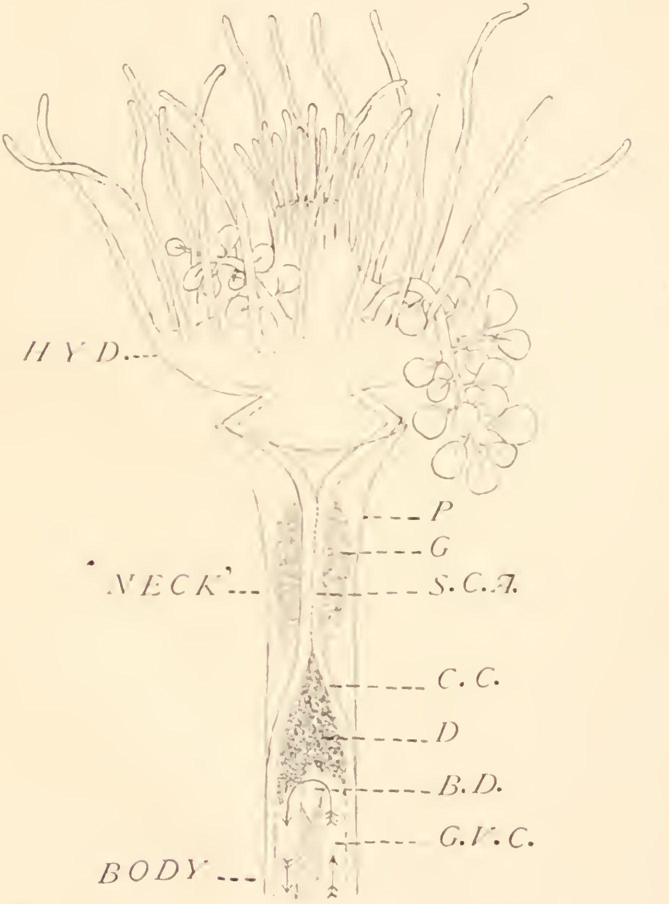
[1] Giard, A., "L'Autotomie dans la série animale." *Revue scientifique*, p. 629, 1887.

I cite the case of a tubularian which was kept mechanically stimulated by touching or pricking the hydranth with a dissecting needle and in which the process of autotomy had advanced at such a rate as readily to separate at the end of four hours when lifted from the sea-water. With the beginning of contraction in this animal the circulatory current in the "neck" region was stopped; indeed the circulatory-nutritive fluids were quite expelled and excluded from approximately two millimeters of this region; the entodermic walls of the tube here being completely and tightly apposed. The closure at this point also largely stopped for a time the circulation through the stem. The fluids, however, were as before in *contact* with the walls of the gastro-vascular cavity everywhere except in the much contracted neck region. That is to say, in the contracted animal the normal nutritive fluids were in contact with all the structures with which they are normally in contact except at one point—the "neck" region; *it is always at this latter point that histolysis and autotomy later occur.*

In a little less than an hour it was found that the dissepiment which divides the gastro-vascular cavity of the stem into two channels—one for the anterior, the other for the posterior flow of the circulatory fluids—had been broken at a point a little below the contracted "neck," and that the usual *circulation* of fluids was again established within the stem. Soon however there accumulated at the point immediately below the neck a quantity of the red pigment and other débris from the circulation; thus the channel became so firmly plugged that even a relaxation of the contracted neck region could not now effect a reëstablishment of circulation in this "neck" region.

It is my opinion, furthermore, that the reëstablishment of this circulation after a few hours of contraction might be prevented or at least greatly hindered by another circumstance if for any cause the débris just mentioned should fail to collect and act as an effective block. I refer to the accumulation of a gelatinous mass between the perisarc and cœnosarc which begins to be secreted by the cœnosarc soon after it pulls away from its contact with the perisarc. The secretion is perhaps of the nature



Representing the condition leading to autotomy in a tubularian. Such conditions are present in *Tubularia* which have been kept stimulated from one to several hours. *Hyd* = hydranth; *B.D.* = break in dissepiment; *C.C.* = cœnosarc or body wall; *D.* = debris left by circulating current; *G.* = gelatinous mass secreted by contracted cœnosarc; *G.V.C.* = gastrovascular cavity of body; *P.* = perisarc; *S.C.A.* = strongly contracted area = "neck."

of material for a new perisarc, and as noted by Godlewski it hardens on contact with water. The accumulation and hardening of this mass would probably make a reopening of the closed channel very difficult or quite impossible.

We see then that the contraction of the contractile parts of *Tubularia* acts differentially upon its various organs. Such contraction does not rob the hydranth of its contained fluids, nor of its ability to circulate these fluids. The same is true for the stem region, except that there the actual movement of the fluids is in abeyance for a very short time. The contraction which occurs in the neck region, however, brings about far different relations between the contracting area and the nutritive medium. Here there results, not only a cessation of the circulation of fluids, but a complete loss of contact with these fluids following the complete closure of the channel; whilst finally the breaking of the dissepiment immediately below the neck region, and the subsequent plugging of the end of the connecting channel with pigment and débris, preclude the possibility that such contracted region may again regain its circulation together with the food it brings. This area then necessarily disintegrates; and the break—the autotomy—necessarily occurs at this the weakest point.

Our conclusion is that autotomy in *Tubularia* is the result of the contraction of the animal; similar but weaker contractions being common and central features in the behavior of the animal. If the contraction be either too strong, or too much prolonged, autotomy will follow. That is to say, if a very slight strain be put upon the "neck" region while its circular fibers are in a state of extreme contraction separation results at once. If the contraction be not so strong, but considerably prolonged, readjustments are effected in the circulation which prevent the ingress of food to the contracted "neck" region. Degeneration now occurs in this region and the break—the autotomy—follows at this same point. There is, then, no great mystery attached to "l'amputation spontanée"; not even a complex organic correlation to direct a watchful and sacrificial neck in severing an offending head from an unoffending body.

The present work has been done at the Zoölogical Station at Naples while occupying a table supplied by the Carnegie Institution of Washington. To the president of the Carnegie Institution, and to the director and assistants of the Zoölogical Station,

I am much indebted. My best thanks are due to Professor Paul Mayer, of the Zoölogical Station, for special apparatus and for very many kindnesses. For other support of this work I am further indebted to the Laboratory of Experimental Therapeutics, The University of Chicago.

Naples,
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Aug. 30, 1915.

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